A STUDY OF SERUM N-TELOPEPTIDE

A BONE RESORPTIVE BIOMARKER IN

POST MENOPAUSAL WOMEN

Dissertation Submitted for

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THANJAVUR MEDICAL COLLEGE,

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THE TAMIL NADU DR. MGR MEDICAL UNIVERSITY

CHENNAI

APRIL - 2013

CERTIFICATE

This is to certify that dissertation titled "**A STUDY OF SERUM N-TELOPEPTIDE - A BONE RESORPTIVE BIOMARKER IN POST MENOPAUSAL WOMEN"** is a bonafide work done by **Dr.P.SUNITHAPRIYA** under my guidance and supervision in the Department of Biochemistry, Thanjavur Medical College, Thanjavur during her post graduate course from 2010 – 2013.

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DECLARATION

I, **Dr. P. SUNITHAPRIYA** hereby solemnly declare that the dissertation titled "**A STUDY OF SERUM N-TELOPEPTIDE - A BONE RESORPTIVE BIOMARKER IN POST MENOPAUSAL WOMEN"** was done by me at Thanjavur Medical College and Hospital, Thanjavur under the supervision and Guidance of my Professor and Head of the Department **Dr. N. SASIVATHANAM M.D (BIO), D.G.O.** This dissertation is submitted to Tamilnadu Dr. MGR Medical University, towards partial fulfillment of requirement for the award of M.D Degree (Branch XIII) in Biochemistry.

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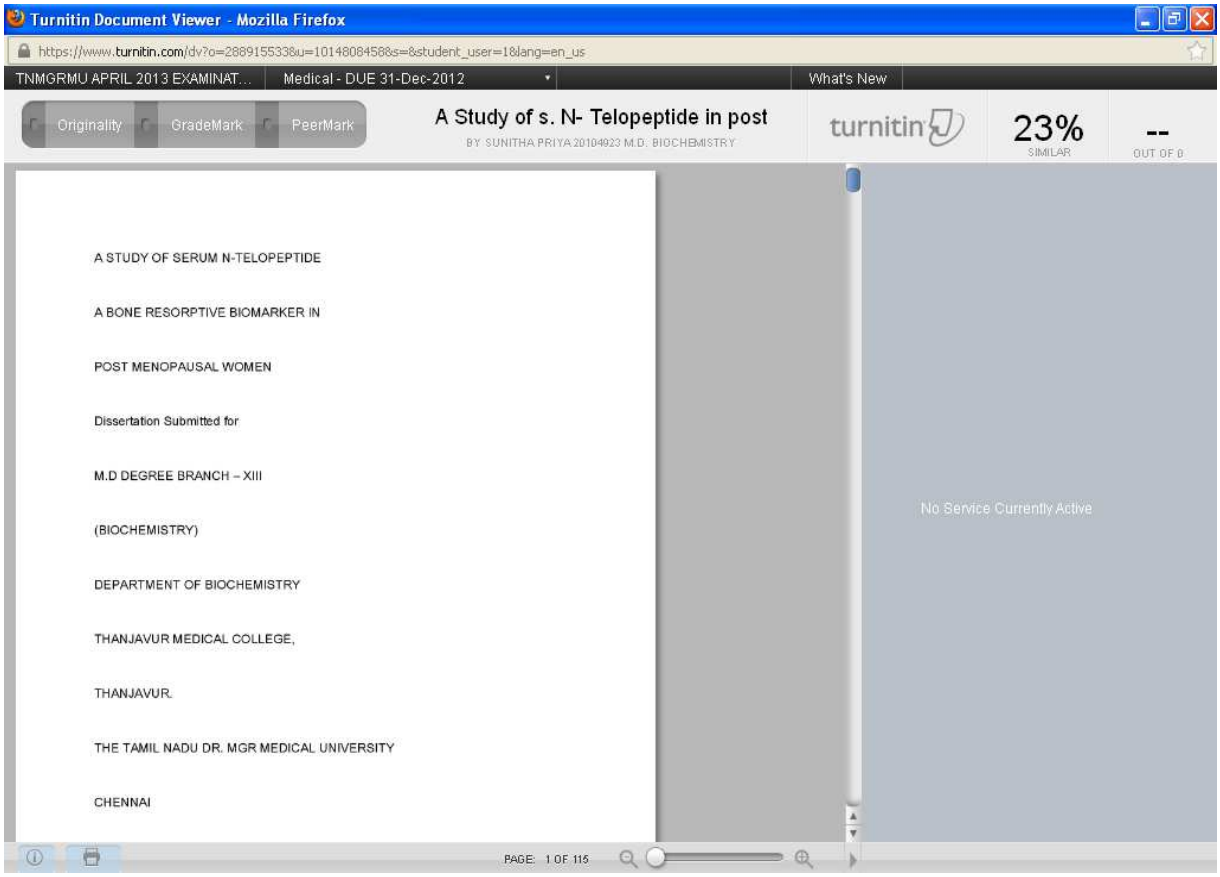

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CONTENTS

ABBREVIATIONS

NTx	-	N-Telopeptide
BMD	-	Bone Mineral Density
IL-1	-	Interleukin - 1
IL-6	-	Interleukin – 6
TNF-α	-	Tumor Necrosis Factor α
RANK	-	Receptor Activator for Nuclear Factor κ B
RANK-L	-	Receptor Activator for Nuclear Factor κ B Ligand
OPG	-	Osteoprotegrin
TGF-β	-	Transforming Growth Factor β
FDA	-	Food And Drug Administration
TRAP	-	Tartrate Resistant Acid Phosphatase
HRP	-	Horse Radish Peroxidase
TMB	-	Tetra Methyl Benzidine
ALP	-	Alkaline Phosphatase

STUDY OF SERUM N-TELOPEPTIDE, A BONE RESORPTIVE BIOMARKER IN POST MENOPAUSAL WOMEN

ABSTRACT

INTRODUCTION:

Osteoporosis is a metabolic bone disorder characterized by low bone mass associated with micro architectural deterioration of bone tissue leading to enhanced bone fragility and an increase in fracture risk. Postmenopausal osteoporosis is characterized by a hormone dependent acceleration of bone loss that occurs after menopause. Serum N-TELOPEPTIDE, a bone resorptive biomarker helps to identify the post menopausal woman with high bone turnover rate and thereby predict their future fracture risk.

AIMS AND OBJECTIVES:

The AIM of the study is to measure S.N-Telopeptide, in postmenopausal women and to evaluate the relationship between N-Telopeptide and S.Estrogen.

MATERIALS AND METHODS:

Study group included 50 postmenopausal women aged 50-70 years who were not previously diagnosed as osteoporosis and who had BMD T-score $\geq$ - 2.5 and T-score between -1.0 to -2.5. Control group include50 premenopausal women aged 25-35 years. S.N-Telopeptide and S.Estradiol were measured by ELISA method.

RESULTS:

The mean range of S.N-Telopeptide in the study group (101.47 nmolBCE/L) is significantly higher than the control group mean (17.35 nmol BCE/L) and the p value is statistically significant. Pearsons correlation studies showed a negative correlation

between N-telopeptide and Estrogen, BMD, BMI and a positive correlation between N-Telopeptide and Total Alkaline Phosphatase which is statistically significant.

CONCLUSION:

This study shows that there is a significant increase in serum N.Telopeptide level in postmenopausal women and it correlates well with the degree of osteoporosis.

KEY WORDS:

N-Telopeptide, Post menopausal osteoporosis, Estrogen, BMD, BMI.

INTRODUCTION

Osteoporosis is a metabolic bone disorder characterized by low bone mass associated with micro architectural deterioration of bone tissue leading to enhanced bone fragility and an increase in fracture risk.[1]

In the new millenium, as we reap the stupendous advances in medical science we are faced with new problems of "ageing". With increase in life expectancy osteoporosis is one of the major public health problem that increases the morbidity and mortality in postmenopausal women. [2]

Osteoporosis is a silent epidemic. Nearly 14% of postmenopausal women and 8% of men above 50 years of age have osteoporosis.[3] The hallmark of osteoporosis is, in its predisposition to fracture following relatively a minor trauma. At the age of 50, women have a life time fracture risk of about 40%.

Postmenopausal osteoporosis is characterized by a hormone dependent acceleration of bone loss that occurs after menopause. In regard to postmenopausal osteoporosis decreased quantity of sex hormone mainly "estrogen" is the prime factor that results in unbalanced bone remodelling. Decreased estrogen level results in increased production of cytokines like IL-1, IL-6 and TNF by blood monocyte and bone marrow cells. These cytokines, in turn, mediates osteoclast recruitment and activity, by increasing the levels of RANK and RANK-L and decreasing the activity of Osteoprotegrin. Compensatory osteoblastic activity occurs, but it does

not keep in pace, leading to osteoporosis.[4, 5]

Early the diagnosis will reduce the morbidity and mortality rate, but the diagnosis of osteoporosis is complicated by the fact that routine x-rays are not sensitive enough to pick up osteoporosis at an early age. It has been estimated that nearly 40% of skeletal bone mass is lost before the x-ray shows the sign of osteopenia. Further, osteoporosis does not reveal any consistent metabolic abnormality detected by standard laboratory tests and certainly, it is very difficult to predict a fracture at a given time in specific person.[6]

Diagnosis of osteoporosis is made clinically by quantifying the bone mineral density. Recently, new bone turnover markers have been used in attempt to get a better insight into bone micro architecture and they appear as a promising tool for defining the skeletal status of post menopausal women.

Bone turn over markers are useful inexpensive tools for studying bone metabolism in population studies and they also predict fracture risk independent of bone mineral density - High bone turnover per se, can disrupt trabecular architecture by increasing the incidence of trabecular perforation and buckling, thus reducing bone strength without necessarily affecting bone mineral density substantially. [7]

N-TELOPEPTIDE is a stable degradation end product from type-1 collagen. In bone type -1 collagen is formed by two α (1) and one α (2) chains stabilised by

the formation of pyridinium cross links, pyridinoline and deoxypyridinoline in the telopeptide region. During bone resorption, type-1 collagen is degraded, yielding smaller fragments like N-Telopeptide, C-Telopeptide, pyridinoline and deoxypyridinoline in circulation making these fragments available as markers of bone resorption.[8]

The present study on serum N-TELOPEPTIDE, a bone resorptive biomarker helps to identify the post menopausal woman with high bone turnover rate and thereby predict their future fracture risk. The N-Telopeptide assay measures N-Telopeptide linked pyridinoline and deoxypyridinoline in serum and the results are expressed as bone collagen equivalents.

REVIEW OF LITERATURE

Osteoporosis is a metabolic disorder characterised by decreased bone mass and increased susceptibility to fractures secondary to trivial trauma. Osteoporosis is characterised by an altered balance between resorption and formation of bone which leads to gradual decline in bone mass and the decrease in the amount of mineralisation of bone. The ratio between mineral and collagen is normal.

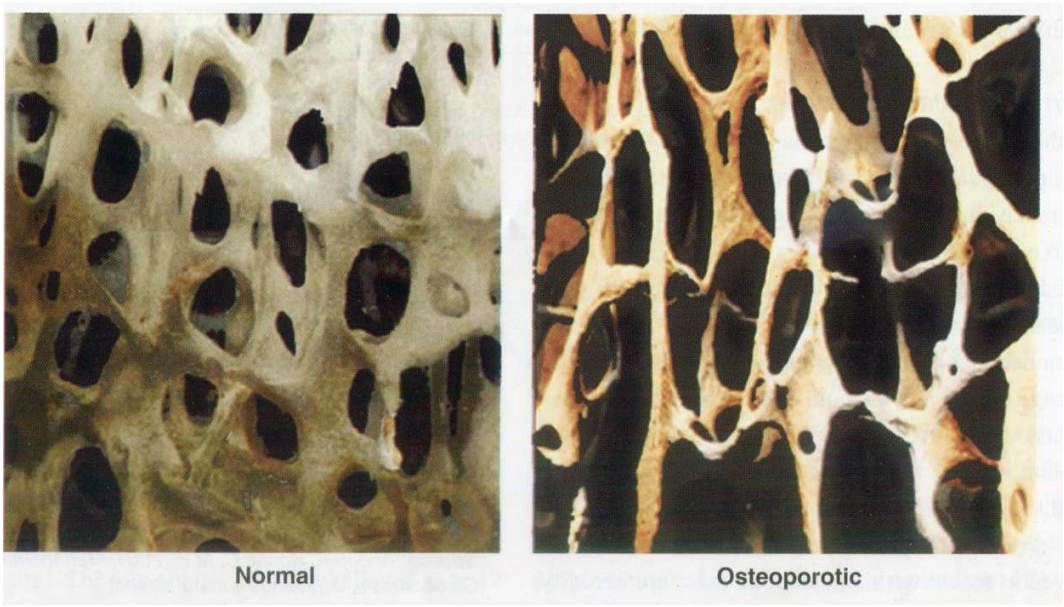
Major consequences of osteoporosis include fragile bones and increased susceptibility to fractures such as

Vertebral compression fracture.

Fracture neck of femur.

Colles fracture.[9]

In early nineteenth century Sir. Astley cooper , a distinguished English surgeon noted-“the lightness and softness that (bones) acquire in the most advanced stages of life” and this state of bone favours much, the production of fractures. Johann lobstein coined the term “osteoporosis”. In 1940, “Fuller Albright” the American physician and endocrinologist described “Postmenopausal osteoporosis” as a sequelae of impaired formation of bone due to deficiency of estrogen. [10]



WHO STUDY GROUP ASSESSMENT OF FRACTURE RISK AND ITS APPLICATION TO SCREENING FOR POST MENOPAUSAL OSTEOPOROSIS.

WHO TECHNICAL REPORT SERIES : 843- 1994 [11]

CATEGORY	**BONE MINERAL DENSITY**	**T SCORE**
Normal	≤ 1SD below average peak BMD of young adult	–
Osteopenia	1.0-2.5 SD below average peak BMD of young adult.	-1.0 to -2.5
Osteoporosis	≥2.5 SD below the average peak BMD of young adult.	≥ -2.5
Severe Osteoporosis	≥2.5 SD below the mean BMD of young adult and history of one or more non traumatic fracture.	≥ -2.5

WORLD HEALTH ORGANISATION describes osteoporosis is a condition in which bone mineral density falls below 2.5 standard deviation(S.D.) from the mean of a young adult of the same sex. It is also termed as T-Score of -2.5.

Now the present concept is, Osteoporosis represents the disorder, in which numerous etiological factors converge resulting in bone loss and breakdown of microarchitectural structure of bone. In osteoporotic patients, various pathogenic factors are added together with increased incidence of falls, contribute to a high incidence of fragility fractures.

EPIDEMIOLOGY:

As our society is ‘‘ageing” there is considerable shift in the epidemiology of Osteoporosis. It is a silent epidemic, but a progressive disease until a fracture occurs. There are 325 million individuals over 65 years of age in this world. This will increase to over 1500 million by 2050.The increase will be particularly seen in Asia.[12]

In developed countries, the fractures related to osteoporosis are estimated to affect 30% of women and 12% of men. The immediate mortality is 12% and there is a continued increase in mortality of about 20% when compared to age matched controls.[13]

WHO TECHNICAL REPORT SERIES – 843 predicts a significant increase in fracture neck of femur among Asian population over a period of time. By 2050, osteoporosis will be a major demographic factor due to changes in life style and the increase in survival rate of elderly.

The incidence of fracture of hip is likely to rise across the world from 8,40,000 in 1986 to approximately 6.26 million in 2050 and 7% of these fractures are likely to occur in the developing countries. India is likely to have a fracture incidence of neck of femur of approximately 1.2 million per year particularly among women.[14]

OSTEOPOROSIS CURRENT SCENARIO IN INDIA

Due to increased life expectancy of Indian population, now it is realised that, as in the west, the osteoporotic fractures are the leading cause of morbidity and mortality in the elderly. In most western countries, the peak incidence of osteoporosis occurs at about 70 – 80 years of age, where as in India it may affect those 10 – 20 years younger at the age of 50- 60 years.

Based on 2001 census, approximately 163 millions of Indians are above the age of 50 and this number is expected to increase to 230 million by 2015. Even conservative estimates suggest that, of these, 20% of women and 10 -15% of men

would be osteoporotic. The total affected population would therefore be around 25 million.[15]

BIOCHEMISTRY OF BONE

Bone consists of organic, inorganic materials and water in percentage composition of 25%, 65%, and 10%

ORGANIC PHASE

- INTERCELLULAR MATRIX (21%)
- BONE CELLS (4%)

INTERCELLULAR MATRIX

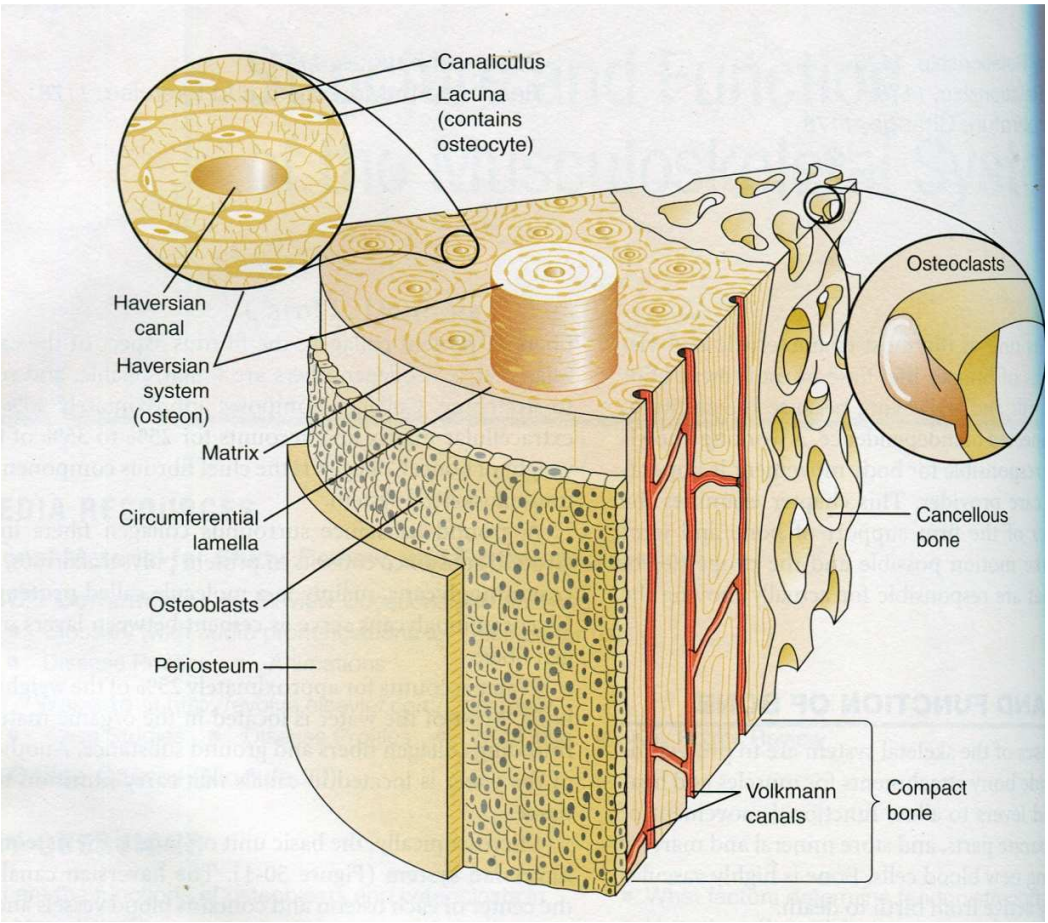
- COLLAGEN
- NON COLLAGENOUS PROTEIN

COLLAGEN

Collagen is the primary extracellular portion of the body consisting of three polypeptide chains in the form of triple helix. One third of the aminoacid composition is glycine, one fifth proline and its derivative hydroxyproline. In addition, it contains hydroxylysine.[72]

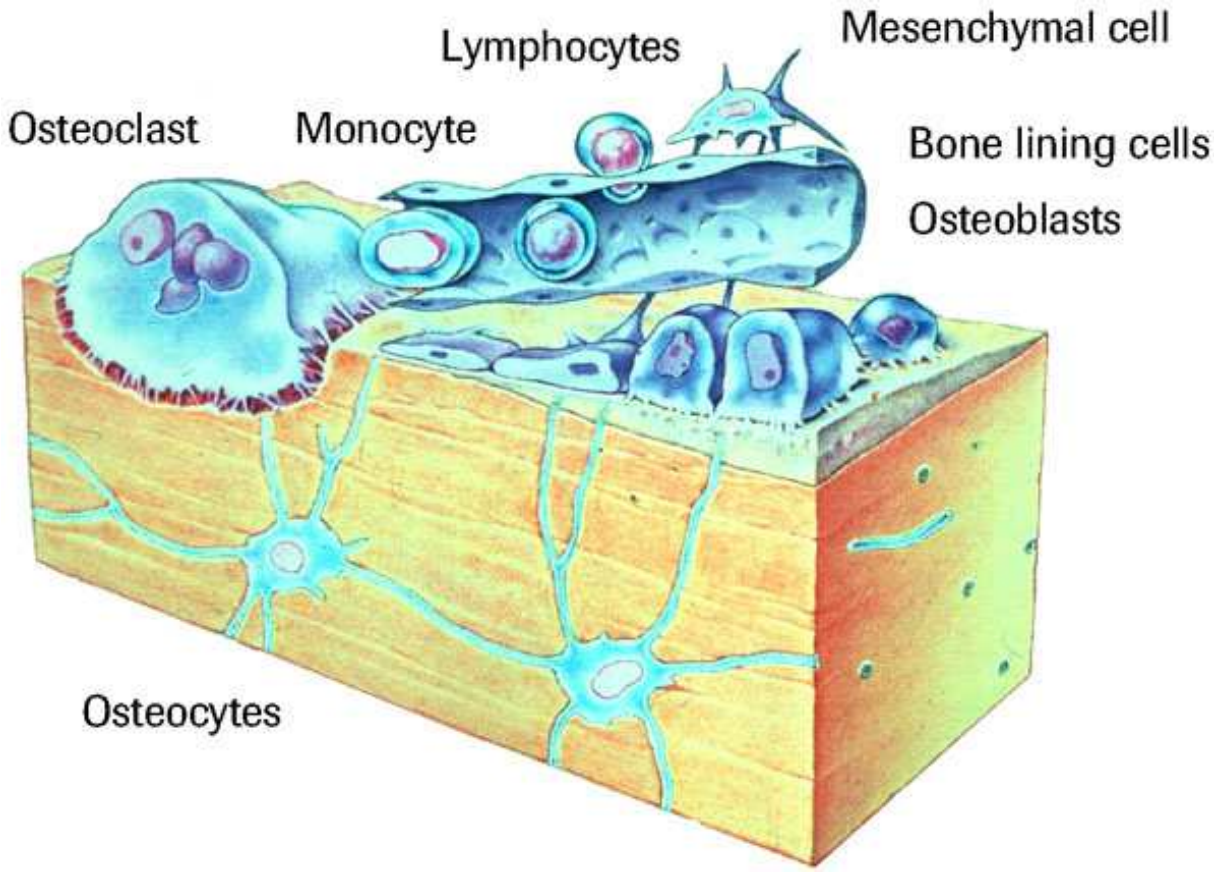
NON COLLAGENOUS PROTEIN

Osteocalcin secreted by osteoblasts constitutes the major non collagenous protein in the bone. It is one of the biomarker of bone formation. Other proteins include Matrix Gla protein and osteonectin.



BONE CELLS :

There are four different types of bone cells having specific functions in bone formation, resorption and remodelling.



OSTEOPROGENITOR CELLS:

Osteoprogenitor cell derived from primitive stem cell differentiates into osteoblasts and osteocytes. They are found in bone marrow, periosteum and other supporting tissues. [16]

OSTEOBLASTS:

The osteogenic cells in the deep layers of periosteum and of the endosteum differentiates in to osteoblasts. Osteoblasts are cuboidal mononuclear cells 15-30 µmm in diameter. They possess an enzyme called Alkaline phosphatase and they have amorphous calcium phosphate. Osteoblasts activate osteoclastic activity. Collagenase secreted by osteoblast is responsible for the attachment of osteoclast at the site of bone resorption either by direct contact or by chemical activity.

They synthesise organic intercellular substance and produce number of growth factors like TGF- β, IL-1, TNF-α and IL-6 which regulate bone growth and differentiation. The osteoblast differentiates into osteocyte in the lacunae of the intercellular substance. [17]

The critical factors involved in activation, proliferation, growth and differentiation of osteoprogenitor cells into osteoblasts include bone morphogenetic protein, basic fibroblast growth factor, insulin like growth factor (I & II), transforming growth factor β 1 & β 2 and platelet derived growth factor.[18]

OSTEOCLAST :

The osteoclasts are exclusively bone resorptive cells and they are multinucleated giant cells varying in size and number of nuclei. They are hematopoietic in origin belonging to monocyte -macrophage family.

The cytokines namely Macrophage Colony stimulating factor (M-CSF) and Receptor Activator of Nuclear Factor –κ B ligand (RANK-L) of Tumor Necrosis Factor super family are produced by the bone marrow stromal cells and osteoblasts. These cytokines are essential for osteoclastogenesis.[19]

INORGANIC MATERIALS:

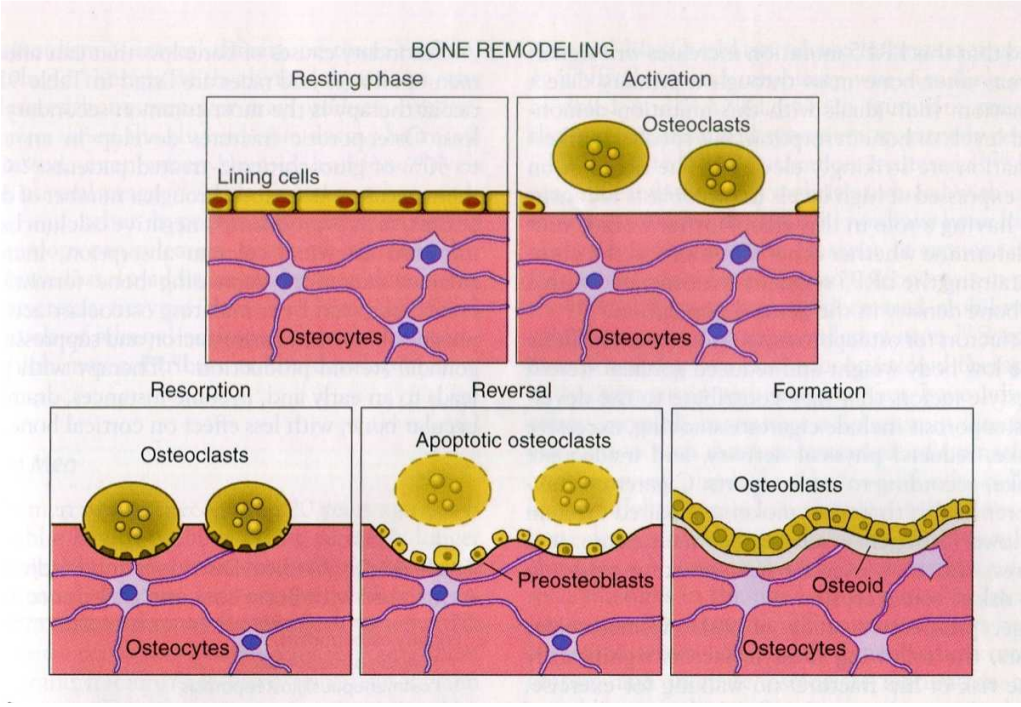
Inorganic phase contains 65% crystalline materials- hydroxy apatite crystals, amorphous calcium phosphate, trapped ions like citrate, flouride, sodium, magnesium and potassium.

WATER:

Water is present either intracellular, extracellular or in bone crystals.

BONE REMODELLING CYCLE:

In adult skeleton, bone remodelling occurs to maintain the skeletal integrity. It is a bone regenerative process that aims to remove the old bone having high mineral density and increased prevalence of fatigue fractures and replace it with new bone with low mineral density and better mechanical properties. [20]



Bone is not a dead tissue, instead the constituent parts are constantly being removed with the whole body turnover rate of 10% per year. Bone is a dynamic structure that undergoes continuous cycles of remodelling consisting of resorption followed by deposition of new bone.[21]

Resorption and formation of bone do not occur randomly in skeleton, but rather takes in bone multicellular units.[22] Bone remodelling cycle usually begins in response to a stimulus –partly in response to the effect of parathyroid hormone which results in multiplication of the precursor cells and the formation of osteoblasts. [23]

Bone remodelling has the following primary functions

- To repair micro damage within the skeleton to preserve the skeletal integrity.
- To regulate serum calcium level by providing calcium from the skeleton via bone resorption.
- To maintain bone mass.

The bone remodelling unit has a characteristic life cycle which is divided into four distinct phases:

- Activation
- Resorption
- Reversal
- Formation

QUIESCENT PHASE:

In adult man, approximately 90% of free bone surface is inactive with respect to remodelling. During quiescent phase, the bone surface is covered by a thin layer of flattened cells. Its function is to protect the bone surface from osteoclastic resorption.[24]

ACTIVATION:

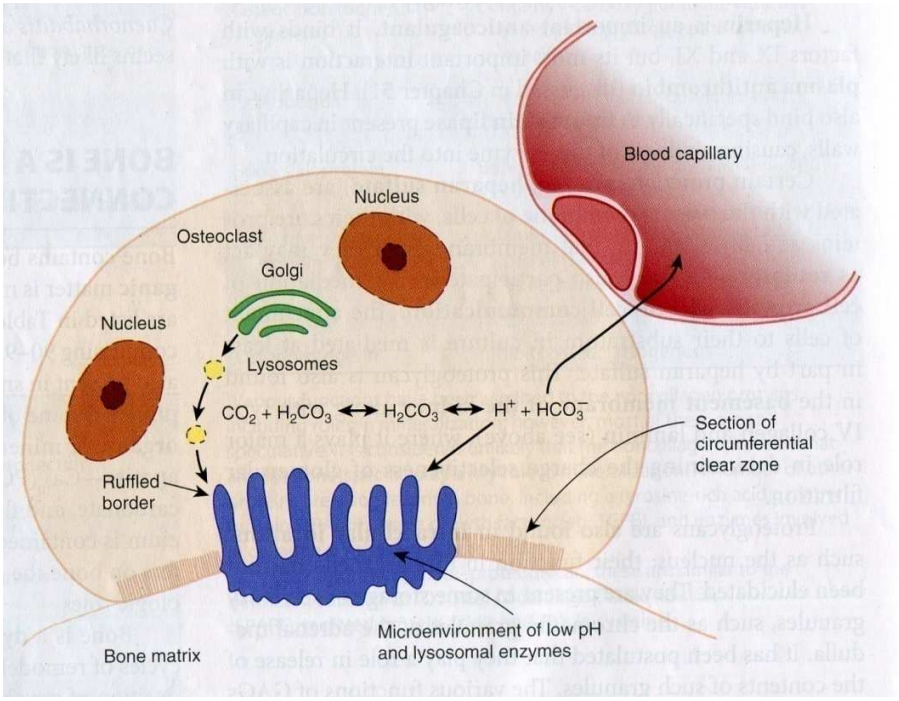
Bone remodelling is initiated by activation of osteoclasts by various systemic hormones like parathyroid hormone, 1,25 –dihydroxy vitamin D_3, sex steroids and glucocorticoids. These hormones indirectly stimulate the osteoclasts by causing the release of factors from osteoblasts like cells.

This is the conversion of small area of bone surface from quiescent phase to resorption phase. In an average adult human skeleton activation occurs approximately every 10 seconds. Osteocytes detect and communicate the mechanical and chemical changes of the surrounding bone matrix to the lining cells on the surface. On receiving signals from these mechanosensor cells lining cells then retract from each other exposing a denuded bone surface that chemoattracts osteoclast precursors. [9]

RESORPTION:

Mature osteoclasts has an apical membrane domain, with ruffled border that play a major role in bone resorption. Across the ruffled border a proton translocating ATPase shuttles the protons in to the resorption area. This reduces the local pH to 4.5or less, thus increasing the solubility of hydroxy apatite crystals and allowing demineralisation to occur.

Local acidic environment promotes the release of lysosomal acid proteases that digest the accesible matrix . [21] When the cavity has reached the depth of 50-100 µm local resorption occurs. Generally bone resorption is rapid and a resorption pit is made within 10-14 days.



REVERSAL PHASE:

During this period, the osteoclastic resorption surface is smoothened by mononuclear cells and a thin layer of mineral rich cement substance is deposited. The osteoblastic precursors assemble prior to bone formation.

FORMATION PHASE :

Bone formation occurs in two stages. Soon after the cementing substance has been deposited, the newly formed osteoblasts begin to deposit a layer of unmineralised bone matrix which is referred to as “osteoid seam”. After deposition, the collagen fibrils make the matrix aggregate and cross link before they become mineralised. The new matrix begins to mineralise after one week. [24]

GENDER DIFFERENCES IN REMODELLING [25, 26, 27]

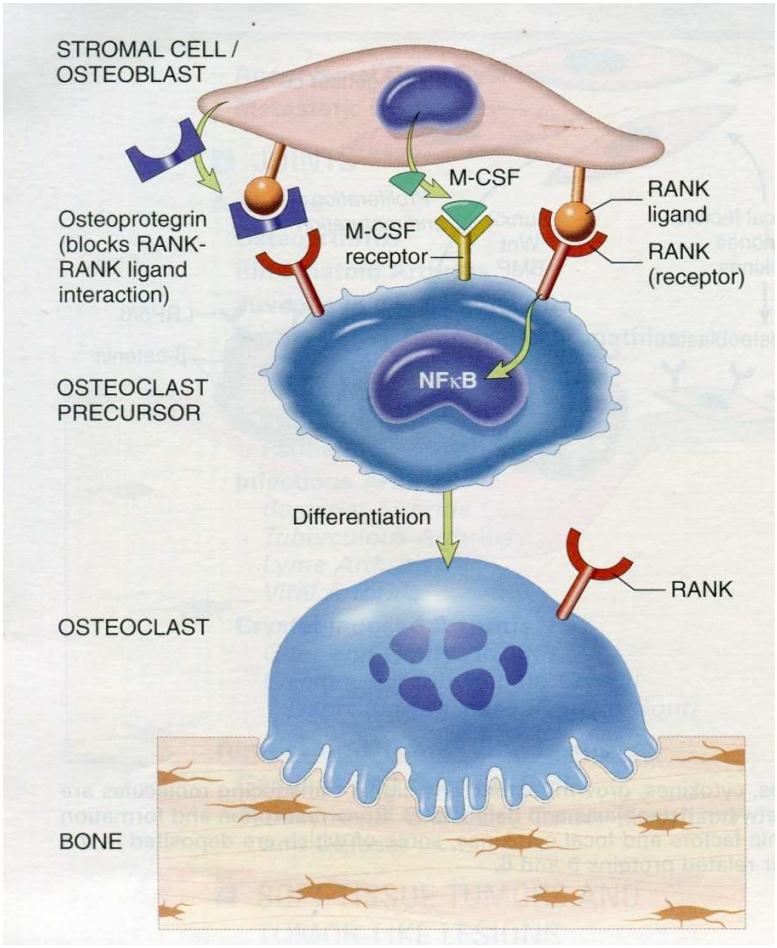
The skeletal architecture in male have a different morphology from female that set them at lower risk for developing osteoporosis. The increased diameter of bone in men provides a greater moment of inertia, which is a strong determinant of torque strength. Additionally, men have greater muscle mass which gives them more agility and padding in case of fall. [25]

In men, ageing results in a gradual decrease in the efficiency with which the osteoblasts refill the resorption cavities. Consequently the thickness of cancellous bone gradually declines which in turn leads to trabecular bone thinning with a linear reduction in the bone volume. Although the individual trabeculae are thinner, the trabecular connectivity is generally preserved.

In female, till menopause there will be gradual thinning of trabeculae and then there is an abrupt acceleration in the rate of bone loss. This accelerated bone

loss persists for about 5-10 years and is accompanied by a dramatic increase in the bone turnover rate. The rapid bone loss experienced after menopause is not the result of osteoblastic insufficiency but rather is due to increased osteoclastic activity.

Incidence of fracture risk varies between men and women and it is due to their difference in their areal BMD, bone size geometry and bone strength.



PARACRINE MOLECULAR MECHANISM THAT REGULATE OSTEOCLAST FORMATION AND FUNCTION

There are several signalling pathways that regulate bone homeostasis.

One such pathway involves

- Transmembrane receptor –RANK (Receptor Activator for Nuclear Factor Kappa B) which is expressed in osteoclasts precursors.
- RANK Ligand (RANK-L) which is expressed in osteoblasts and marrow stromal cells.
- Osteoprotegrin (OPG), a secreted decoy receptor by osteoblasts that can bind RANK-L and thus short circuits its interaction with RANK.

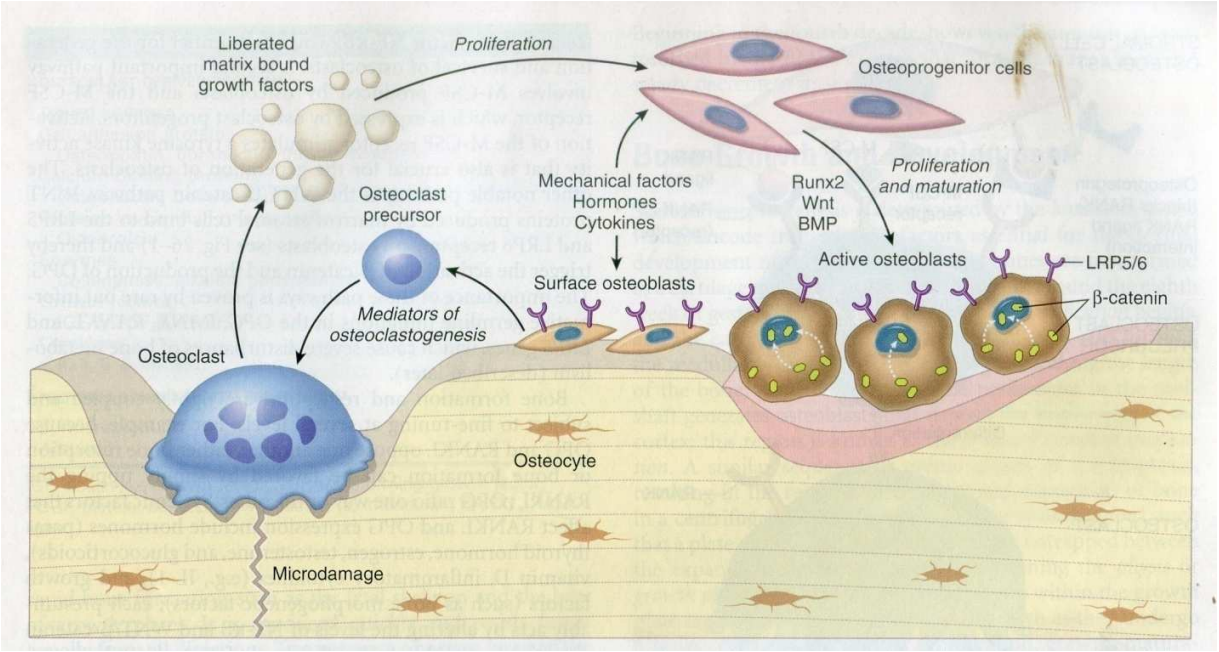
When stimulated by RANK-L, RANK signalling activates the transcription factor, Nuclear Factor kappa B which is essential for generation and survival of osteoclasts. [28, 29]

A second important pathway involves M-CSF produced by osteoblasts and M-CSF receptor expressed by osteoclast progenitors. Activation of M-CSF receptor stimulates tyrosine kinase activity that is crucial for generation of osteoclasts.

The other notable pathway involves WNT/ β Catenin pathway.

WNT proteins produced by marrow stromal cells bind to LRP-5 and LRP-6 receptor (LDL receptor related protein 5 & 6) on osteoblasts and triggers the activation of β-Catenin and the production of Osteoprotegrin.[4]

Activating mutations of the low density lipoprotein receptor related protein 5 leads to high bone mass, where as inactivating mutations leads to osteoporosis psudoganglioma syndrome, a condition leading to low bone mass in children.[30]



ROLE OF ESTROGEN IN BONE METABOLISM

Estrogen has three fundamental effects on bone metabolism:

- It inhibits the initiation of new basic multicellular units and the activation of bone remodelling.
- It reduces bone resorption by inhibiting the differentiation and activation of osteoclast and promotes apoptosis of osteoclasts.
- It stimulates the commitment and differentiation and prevents apoptotis of osteoblastic cells, thereby maintaining bone formation at the cellular level.[31]

In bone metabolism, estrogen mediates its effects through two estrogen receptors. Like other steroid hormones, the receptors for estrogen are protein molecules present in the cytosol. These receptors are ligand dependent transcription factors. Upon ligand binding, they dimerize and translocate in to the nucleus where together with variety of transcription factors they induce or repress the transcription of genes.[26]

There are two different estrogen receptors

(i) Estrogen receptor α –classic receptor

(ii) Estrogen receptor β

Estrogen receptor α and β are present in most of bone cells with estrogen receptor α expression prominent in cortical cells and estrogen receptor β in trabecular cells.[32]

Estrogen receptor-related receptor α (ERR -α) is seen in bone cells with sequence homology to ER α and ER β. This receptor is an orphan nuclear receptor. Despites its inability to interact with estrogen, this receptor binds with Erα /Erβ or act directly to alter bone cell function.[10]

Estrogen exerts direct antiosteoclastic effects at several stages of osteoclastic differentiation and function. Estrogen directly inhibit the formation of multinucleated osteoclasts by suppression of RANKL induced activation of precursor cells.

Estrogen binds to the receptor on osteoclast and downregulate bone resorption by osteoclasts by altering the activity of lysosomal enzymes. Estrogen also affects the ion transport in osteoclast, mainly involving the inward rectifying K^+ channel at the plasma membrane of the osteoclasts leading to depolarisation of plasma membrane which in turn reduces the proton secretion in to the resorption lacunae and thereby inhibit resorption.[32]

Estrogen also increases TGF-β leading to osteoclast apoptosis and increases Osteoprotegrin which blocks the formation and activity of osteoclast.[33] Further,

estrogen blocks the synthesis of proinflammatory cytokines by bone marrow and bone cells.[34]

Thus, Estrogen negatively regulates the number and activity of osteoclast and the loss of this direct function potentially play a role in postmenopausal osteoporosis.

CLASSIFICATION OF OSTEOPOROSIS

On the basis of modified classification by Nordin (1964) which takes in to the account of the findings by WHO –osteoporosis can be generalised or localised.

GENERALISED OSTEOPOROSIS

Primary osteoporosis

Secondary osteoporosis

PRIMARY OSTEOPOROSIS

TYPE-I PRIMARY POSTMENOPAUSAL OSTEOPOROSIS

Postmenopausal osteoporosis which occurs due to loss of ovarian function, reflects an absolute acceleration in the rate of bone resorption due to increased osteoclast recruitement. [35]

Estrogen deficiency primarily affects the trabecular bone than the cortical bone because the former has considerably larger surface area (80% of the total area). The loss of trabecular connectivity associated with increased turn over after menopause may be responsible for bone fragility. [25]

TYPE-II SENILE OSTEOPOROSIS

Type-II senile osteoporosis occurs both in men (65-70 years) and in women (50 years). Patients with senile osteoporosis have accelerated bone loss of 2% per year, with symptoms of osteoporosis beginning when 30% of bone is lost.[36] Loss of both cortical and trabecular bone occur in senile osteoporosis with increased risk of fracture hip, pelvis and vertebra.

SECONDARY OSTEOPOROSIS [37, 38]

MEDICATIONS

1.Glucocorticoids

2.GnRH agonist

3.Loop diuretics

4.Methotrexate

5.Heparin

6.Thyroxine (over replacement)

HEREDITARY / SKELETAL / CONNECTIVE DISEASES

1.Osteogenesis imperfecta

2.Rickets

3.Hypophosphatesia

4.Marfans syndrome

ENDOCRINE AND METABOLIC CAUSES

1.Hypogonadism

2.Hyperthyroidism

3.Hyperparathyroidism

4.Cushing's syndrome

5.Gaucher's disease

6.Hemochromatosis

7.IDDM

MARROW DISEASES

1.Myeloma

2.Mastocytosis

3.Thalassemia

4.Leukemia

RHEUMATOLOGICAL DISORDERS

1. Systemic lupus erythematosis
2. Ankylosing spondylitis
3. Rheumatoid arthritis

MISCELLANEOUS

1. Renal insufficiency
2. Chronic obstructive pulmonary disease
3. Anorexia nervosa
4. Malnutrition
5. Malabsorption
6. Cystic fibrosis
7. Coeliac disease
8. Peptic ulcer
9. Crohn's disease
10. Breast cancer treated with aromatase inhibitors.

LOCALISED SECONDARY OSTEOPOROSIS

1. Prolonged immobilisation
2. Monoarticular Rheumatoid arthritis
3. Sudeck's osteodystrophy.

PRIMARY POSTMENOPAUSAL OSTEOPOROSIS [39, 40]

Postmenopausal osteoporosis is an exaggerated form of physiological bone depletion that normally accompanies ageing and a loss of gonadal activity. Two overlapping phases are recognised.

HIGH TURN OVER OSTEOPOROSIS

Early postmenopausal syndrome characterised by rapid bone loss predominantly due to increased osteoclastic resorption.

LOW TURN OVER OSTEOPOROSIS

This is a less well defined syndrome which emerges in elderly people due to gradual slow down of osteoblastic activity and the increasing effects of dietary insufficiency, chronic ill health and reduced mobility.

PEAK BONE MASS

Bone formation continues at pace faster than bone resorption until maximum bone strength is reached around the age 30.[41] Peak bone mass can be defined as highest level of bone strength achieved in an individual following normal growth.

Factors determining peak bone mass include

- Genetic
- Hormonal
- Environmental
- Mechanical loading. [42]

Ageing is the major risk factor, because after 35-40 years of age, bone resorption slightly exceeds bone formation so that approximately 1% of skeletal bone is lost per year. In women the decrease in sex steroids at menopause accelerates the bone loss to about 2% per year for the first five years and then declines to 1% loss per year.[43, 44]

RISK FACTORS FOR POST MENOPAUSAL OSTEOPOROSIS

GENETIC FACTORS

1. Family history of osteoporotic fractures.

2. Whites > blacks.

3. Absence of generalised osteoarthritis

ANTHROPOMETRIC

1. Small statured.

2. Fair thinned pale skin.

3. Thin body habitus.

HORMONAL FACTORS

1. Women > men

2. Early menopause and late menarche

3. Nulliparity

DIETARY

1. Low dietary calcium.

2. Excess protein.

LIFE STYLE CHANGES

1. Sedentary

2. Smoking

3. Alcoholism.

CONCURRENT ILLNESS AND DRUGS

1. Gastrectomy.

2. Hyperparathyroidism.

3. Hyperthyroidism.

4. Rheumatoid arthritis.

5. Cushing ‘s syndrome.

6. Corticosteroid therapy.

7. Neurological diseases-Parkinsonism

8. Cerebro vascular accidents [45, 46]

GENETIC CAUSE:

Genetic osteoporosis refers to the concept that osteoporosis is frequently a manifestation of genetic disorder. Hereditary plays a role by determining the peak bone mass that a woman will attain during her life. African and American women have high bone mass than white women and this explains the low risk of osteoporotic fractures observed in African-American women. [47]

Numerous genes regulate skeletal growth, peak bone mass, body size while others control skeletal structure and density. The candidate genes include those encoding collagen type 1α 1,estrogen and vitamin D receptor, TGF-β, insulin like growth factor, apolipoprotein E, IL-6 and bone morphogenetic protein. The effects of these genes are hormonally modulated, which are themselves genetically regulated.[48]

A polymorphism in SP1 binding site of collagen α-1 gene promoter is associated with the phenotype of low bone density and increased propensity to fracture in old age without the more severe manifestations of classical osteogenesis imperfecta.

MECHANISM OF OSTEOPOROSIS MEDIATED BY COL1A1 SP1 POLYMORPHISM

The COL1A1 SPI T allele has increased affinity for SPI binding and the COL1A1 mRNA abundance is increased. This causes an imbalance in the ratio of α1 and α2 chains probably resulting in collagen 1 α1 homotrimer this adversely affects the mineralisation, reduces bone strength and predisposes to osteoporotic fracture. [33]

Linkage analysis suggests that there multiple genes involved in osteoporosis.

- G to T polymorphism in the regulatory region of COL1A1 gene is significantly related to bone mass and risk of osteoporotic fractures in postmenopausal osteoporosis.
- Early onset osteoporosis is associated with mutations in COL1A1 gene on 17q and COL1A2 gene on 7q.
- Polymorphisms in genes coding for vitamin D receptor and calcitonin receptor (CALCR) also contribute to genetic risk for osteoporosis.[49]

High serum homocysteine levels have adverse effects on bone methyl tetra hydro folate reductase which in turn affects methylation of homocysteine to methionine and may negatively affect bone density leading to osteoporosis.[50]

SMALL STATURE AND THIN BUILT HABITUS

Thin built women are at higher risk than obese women, particularly of their low estrogen production, their lower concenteration of circulating estrogen, and the decreased mechanical stress on their bone.[51]

HORMONAL CHANGES

During Postmenopausal period estrogen deficiency is due to either waning of ovarian function or amenorrhoea. The rapid bone loss experienced after menopause is due to enhanced osteoclastic activity and an overall increase in the bone remodelling. IL-6and TNF are the main causative agents underlying bone loss induced by estrogen deficiency, mainly by upregulating osteoclast formation and activation.

Following menopause there is an accelerated increase in the production of IL-1 and TNF by the monocyte which in turn stimulates stromal cells in the bone marrow or their osteoblast progeny to release factors like IL-6, IL-1, Granulocyte Macrophage Colony Stimulating Factor, Monocyte Macrophage Colony

stimulating Factor and RANK-L. These factors in turn stimulate proliferation of hamatopoietic osteoclastic precursor cells originating from cells of Granulocyte/Macrophage colony forming unit and Monocyte /Macrophage colony forming unit lineage to form mature active osteoclasts.

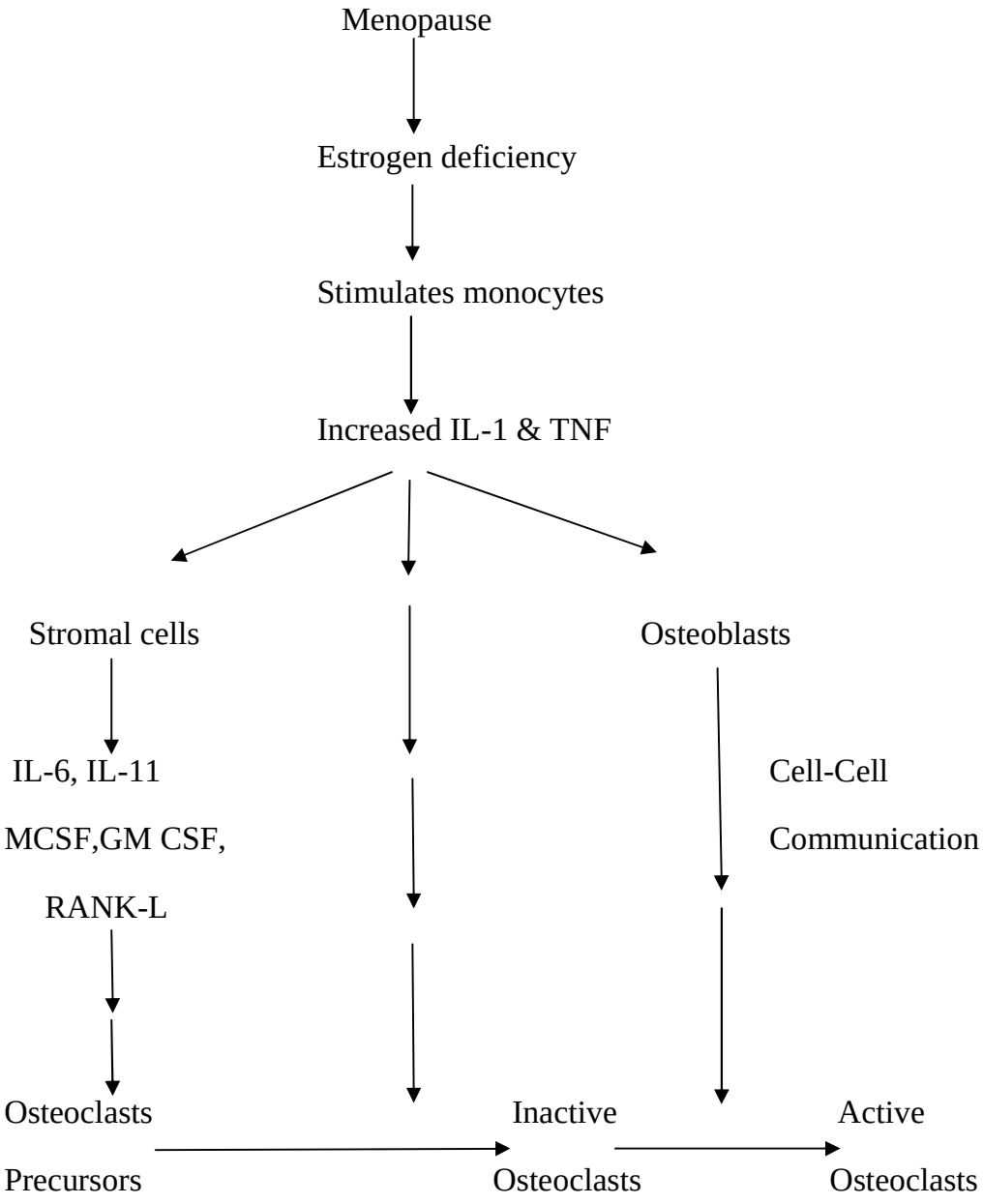
An increased number of resorption sites are active per unit time and the osteoclast dig further into trabeculae and often perforate and further remove them. This is due to increase in the life span of osteoclast as a result of decreased estrogen level. [48]

Estrogen normally acts as a block to the second messenger from the osteoblast to the osteoclast when the former is stimulated by parathormone. Thus estrogen deficiency indirectly increases the sensitivity of osteoclasts to Parathyroid hormone. [1]

Estrogen normally exerts its beneficial effects by suppressing “Reactive oxygen species”. As the Thiol antioxidant defence mechanisms are decreased in estrogen deficiency there is a resultant increase in reactive oxygen species may induce TNF-α. [10] Estrogen deficiency also results in the intrinsic gut wall defect in calcium absorption .

Thus in postmenopusal women decreased estrogen level via activation of various growth factors and inflammatory cytokines affect osteoblastic and osteoclastic activity results in negative bone balance and finally osteoporosis.

MENOPAUSE AND OSTEOPOROSIS



DIETARY CAUSES

LOW CALCIUM LEVEL[52]

Though the principle cause of age related osteoporosis is the continuing effects of estrogen deficiency, a further contribution is also provided by “calcium deficiency”.

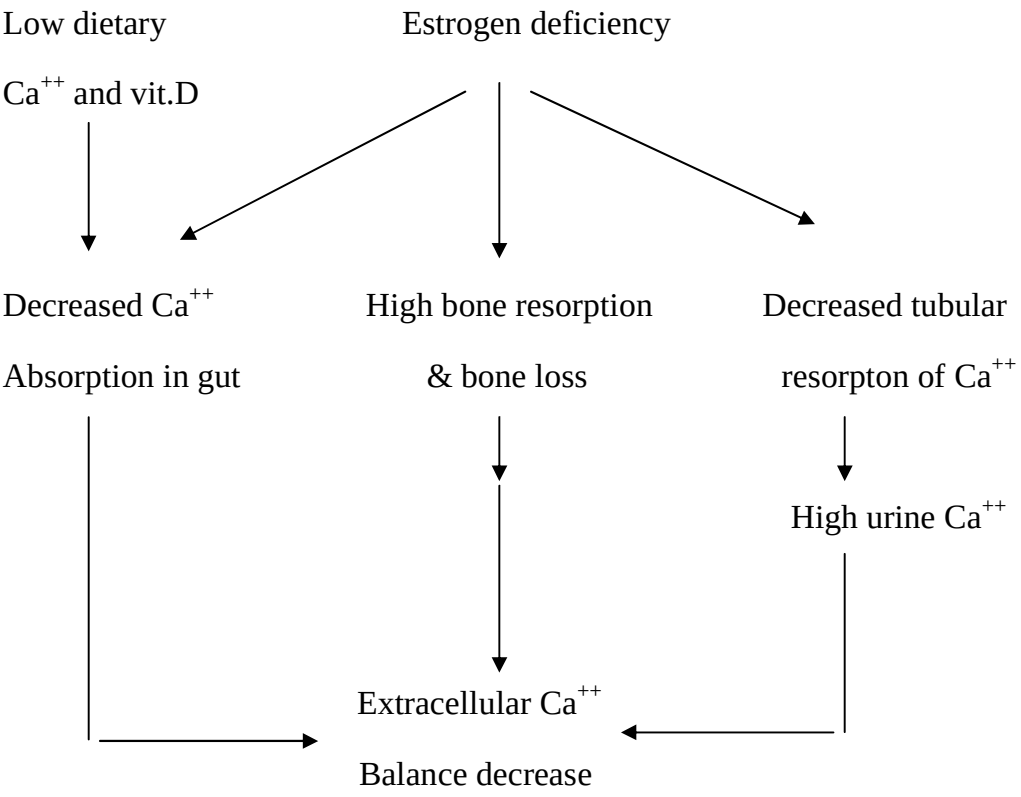
The cause of calcium deficiency is complex and is related to age related decrease in calcium absorption in the gut and a menopause related acceleration in renal calcium excretion.

The intestinal absorption of calcium is decreased mainly due to reduced effectiveness of vitamin D on stimulating calcium absorption. This in turn is due to decrease in the precursor of calcitriol, 25 OH vitamin D, due to lack of sunlight and of calcitriol itself, due to renal impairment.

The renal excretion of calcium is increased due to decreased resorption of calcium in the distal convoluted tubule associated with the loss of estrogen stimulation of Ca^{++} ATPase. [53].

The net effect of these two process increases bone resorption via stimulation of osteoclastic activity, which in turn is mediated in part by a rise in parathyroid hormone level.

ROLE OF CALCIUM IN OSTEOPOROSIS



OTHER NUTRITIONAL FACTORS

LOW PROTEIN INTAKE

Decreased protein intake has serious effects in the elderly. Malnourished individuals with inadequate protein intake are frequently associated with hip fractures. Supplementation of diet with protein has been found to reduce complications in hip fracture.[9]

In elderly individuals decreased phosphate intake may contribute to bone loss. In chronically ill patients or in those who have malabsorption syndrome low magnesium levels also accelerate bone loss.

LIFE STYLE CHANGES

CIGARETTE SMOKING

Cigarette smoking for a long period has detrimental effect on bone mass and affects the bone mass. These effects are mediated either directly by its toxic effects on osteoblasts or indirectly by modifying estrogen metabolism. On an average, cigarette smokers normally attain menopause 1-2 years earlier than the general population.

Cigarette smoking also produces secondary effects that can modulate skeletal status like

- intercurrent respiratory illness
- decreased exercise
- poor nutrition
- the need for additional medications.[48]

ALCOHOLISM

Alcoholism is the common cause of osteoporosis at all ages, with added factor of increased tendency to falls and other injuries. Bone changes seen in alcoholism are due to combination of decreased calcium absorption, liver failure and toxic effects of alcohol on osteoblast function. [39]

INADEQUATE PHYSICAL ACTIVITYAND SEDENTARY LIFE STYLE

WOLFF'S LAW (LAW OF TRANSFORMATION OF BONE) [54]

WOLFF 'S LAW describes the relationship between bone geometry and mechanical influences on the bone and the response of bone to stress. Following increased load on the particular portion of the bone, the bone will remodel itself over time to become stronger to resist that sort of loading. The internal architecture of trabeculae undergoes adaptive changes followed by secondary changes to the external cortical portion of the bone which becomes thicker .The converse is true – if the loading on the bone decreases ,the bone becomes weaker since there is no stimulus for remodelling.

Thus inadequate physical activity and immobilisation results in profound bone loss and osteoporotic fractures are aggravated.

PATHOPHYSIOLOGY OF POSTMENOPAUSAL OSTEOPOROSIS

The bone strength in an elderly woman is the product of her peak bone mass and bone loss .Cessation of estrogen production after menopause results in disturbance between bone resorption and bone formation such that the amount of bone removed by osteoclast exceeds the rate of new bone formation by osteoblasts.

As age advances remodelling continues in the same intensity, but due to estrogen deficiency the extent of coalescence of pores increases, so the number of pores in the cortical bone decreases but the total area of porosity increase. Cortical porosity reduces the ability of the bone to limit crack propagation so that the bone cannot absorb the energy imparted by fall and this energy is released in the most undesirable way, by fracture.[55]

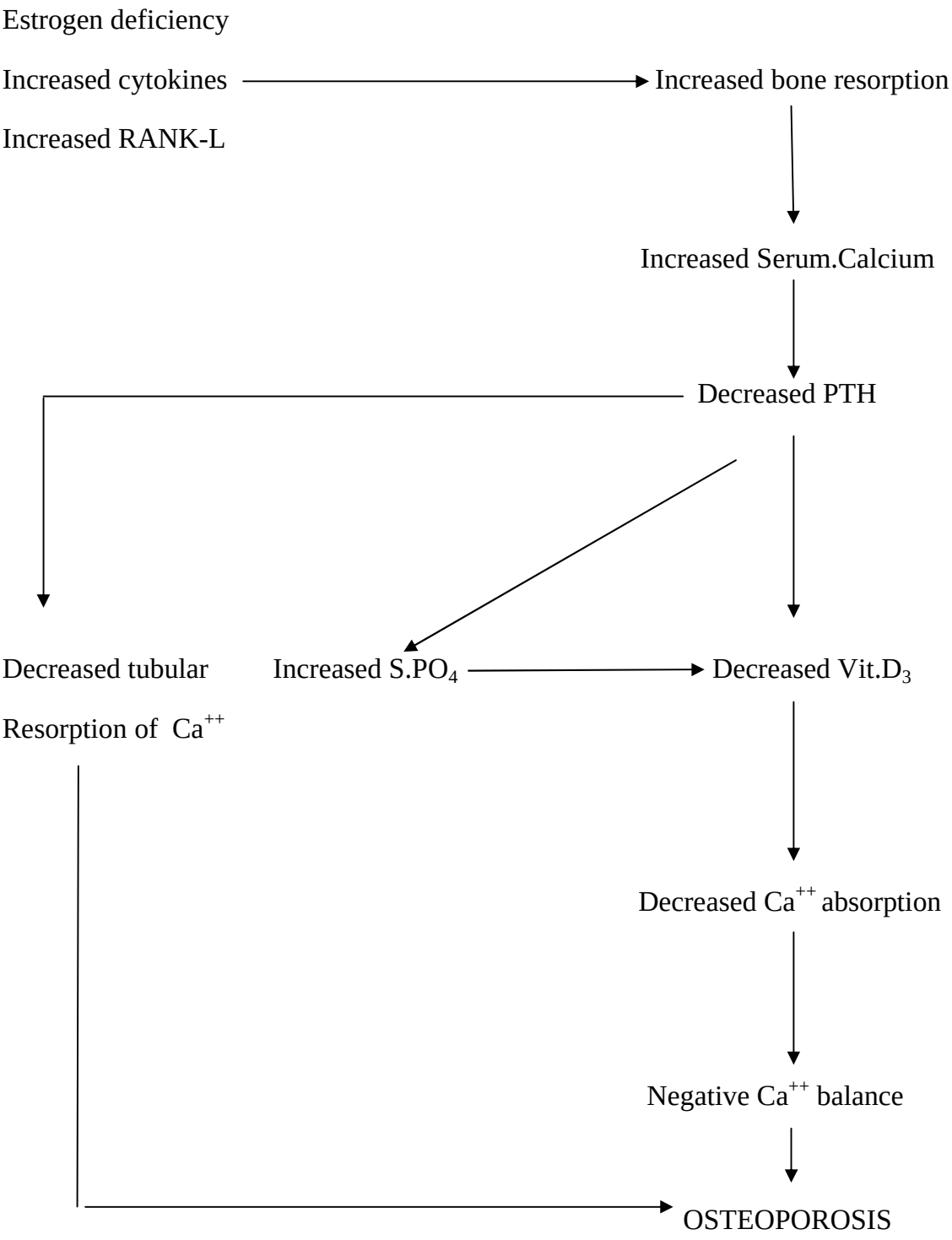
In the bone marrow accumulation of fat occurs because of age related decline in the ability of bone marrow cells to differentiate into osteoblast and an increased ability to differentiate into adipocytes. [13]

The characteristic radiological features in osteoporosis is thinning of cortical bone and generalised rarefaction of skeleton. The increased osteoclastic activity

mainly affects the bone with increased surface area like cancellous compartment of vertebral bodies. In vertebral column there is thinning and disappearance of transverse trabeculae followed by thinning of vertical trabeculae finally leading to compression fractures and widening of inter vertebral disc resulting in the characteristic “FISH MOUTH APPEARANCE”. The lumbar and thoracic vertebrae are most commonly affected. [56]

Radiological surveys have shown that 20% of men and 30% of women over age 60 have compression fractures of vertebral bodies. Back pain associated with height loss and thoracic kyphosis characterised by Dowager’s hump are common manifestations.

PATHOGENESIS OF PRIMARY POST MENOPAUSAL OSTEOPOROSIS

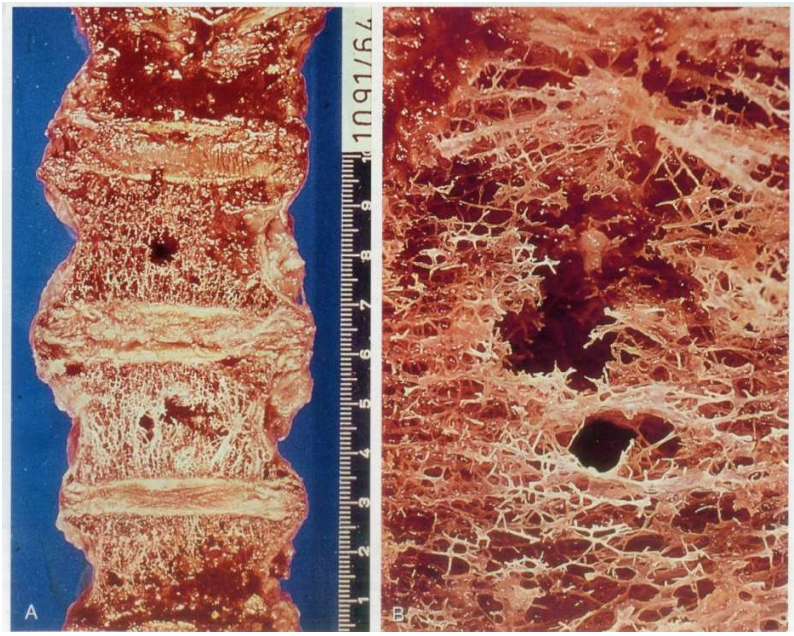


POSTMENOPAUSAL OSTEOPOROSIS- DIAGNOSIS

Most of the cases of osteoporosis are not diagnosed, until a fracture occurs. A routine spinal radiograph in a symptomatic patient will not detect osteoporosis until 40% of bone is lost. Hence a radiograph is not useful in the follow up of progression of bone loss in osteoporotic patients. [57] Early diagnosis and intervention may produce beneficial results.

The diagnosis of osteoporosis should

- Confirm the presence of osteoporosis
- Rule out secondary causes of osteoporosis
- Establish a baseline against which patient's progress can bemonitored.



METHODS TO DIAGNOSE OSTEOPOROSIS [58]

- Measurement of Bone mineral density
- Laboratory biochemical markers
- Bone biopsy with pathological evaluations

The commonly used technique to diagnose osteoporosis and predict future fracture risk is assessment of BMD by bone densitometry which is principally a measure of mineral content of bone.BMD measurements strongly correlate with load bearing capacity of hip and spine and also with the risk of fracture.

Bone density evaluation usually incorporates the attenuation of soft tissues and bone by x-rays to calculate the BMD. Usually this technique compares the patient's BMD with age and sex matched controls (z-score) and with young adults (T-score).

The values of bone mineral density is reported either as an absolute value and in terms of "T-Score" which represents the number of standard deviations (S.D.) from the young normal mean BMD. For every one SD decrease in BMD, the relative risk of fracture approximately doubles. [59]

NATIONAL OSTEOPOROSIS FOUNDATION (NOF) recommends that a women should be given a bone density test if they meet the following criteria[61]

- Older than 65 years.
- Postmenopausal with at least one risk fracture besides menopause or with fracture.
- Considering osteoporosis therapy.
- On prolonged hormone replacement therapy.

Since bone quality cannot be measured clinically, calculating osteoporosis with low dose x-rays is used for assessing fracture risk in osteoporosis.

TECHNIQUES FOR MEASUREMENT OF BMD [60, 43]

TECHNIQUE	COMMENTS
Conventional skeletal radiography	Insensitive method. Detects when bone mass loss is greater than30%.
Photon absorptiometry	Requires radionucleide source of photons.
Single energy x-ray absorptiometry	Used in peripheral sites. Cannot be used in axial skeleton.
Dual energy x-ray absorptiometry	Preferred method. Measures total body mineral density.
Computed tomography	Used for forearm and spine. High cost and high radiation dose.
Ultrasonography	Used for cancellous bone in heal. Advantages are low cost, no radiation and easily portable

CONTRAINDICATIONS FOR BONE DENSITOMETRY

The contraindications include pregnancy, recent gastrointestinal contrast studies and radionuclide tests. The results may not be accurate when osteoporosis is associated with osteomalacia, osteoarthritis, vascular calcifications and in the presence of previous fractures.[11]

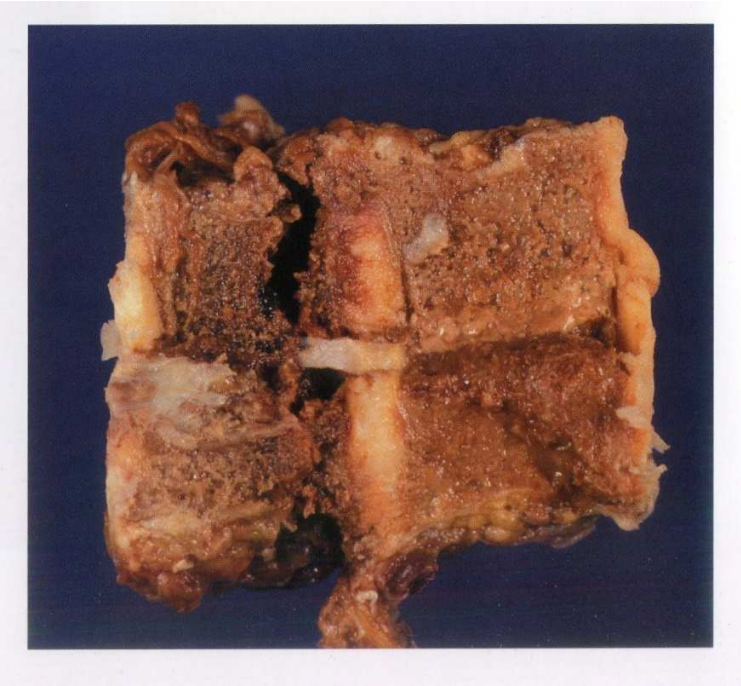
ULTRASONOGRAPHY

Ultrasonography is the technique that provides information about the mechanical properties of the bone like bone density and bone elasticity which are strong predictors of bone strength.

Techniques used in ultrasound include speed of sound and broad band attenuation methods. Speed of sound technique measures bone density and elasticity, where as broad band ultrasound attenuation technique measures bone density, structure and composition. FOOD AND DRUG ADMINISTRATION (FDA) has approved the ultrasound technique to discriminate between normal and osteoporotic individuals who are at increased fracture risk.[59]

However, the densitometric scan do not reflect the dynamic nature of bone tissue. In contrast, biochemical markers of bone turnover provide a better insight of bone growth, bone remodelling and their measurement and hence they are useful in

the assessment of metabolic bone diseases like osteoporosis and they provide an integrated evaluation of global disease activity rather than assessing a regional activity as with densitometric scan.[62]



BIOCHEMICAL MARKERS OF BONE TURNOVER

The biochemical bone turn markers reflect the overall rate of bone formation and bone resorption and has markedly improved the non invasive assessment of bone turn over in metabolic bone diseases like osteoporosis. [63]

Metabolites of bone remodelling which are present in blood and urine serves as an effective markers of bone turnover. They appear as a promising tool for defining the skeletal status of postmenopausal women.

IMPORTANCE OF BONE TURN OVER MARKERS [64]

- Used in the assessment of bone loss

  A sharp increase in the level of bone mass with negative correlation with bone mineral density is associated with increase bone loss. Hence it identifies the disease status before overt damage.

- For assessment of fracture risk [65]

  Increased bone resorption is associated with increased risk of vertebral and peripheral fractures independent of BMD.

- For monitoring the treatment response [66]

  Antiresorptive therapies such as estrogen, calcitonin and bisphosphonates produce a significant decrease in markers to premenopausal range.

- Bone turnover markers helps to identify fast bone losers from slow bone losers.[67]

- In addition to their use in metabolic bone disease, bone turnover markers are potentially useful tools in the diagnosis and monitoring of metastatic bone diseases like Prostatic cancer.[68]

- Further the assessment of changes in the levels of markers of bone turn over will be a useful tool to improve patient persistence.[69]

CLASSIFICATION OF BONE TURN OVER MARKERS

Biochemical markers of bone turn over are classified as bone formation markers which are formed from osteoblast and bone resorption markers produced by osteoclasts.

BONE FORMATION MARKERS [70]

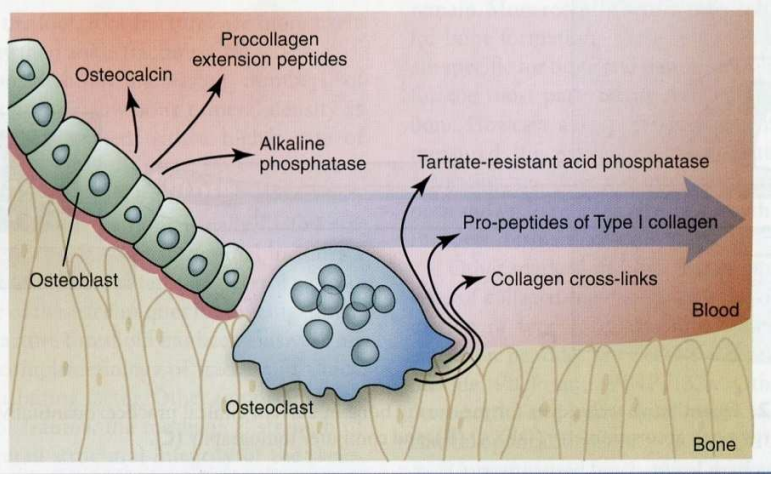
- Alkaline phosphatase (Bone isoform)
- Osteocalcin (bone Gla protein)
- Procollagen type 1 propeptides
- Bone sialoprotein

BONE RESORPTION MARKERS [44]

- Telopeptides
  - N-Telopeptides
  - C-Telopeptides
- Pyridinium cross links
  - Free deoxy pyridinoline
  - Free pyridinoline
  - Total deoxypyridinoline and pyridinoline
- Tartrate resistant acid phosphatase

- Hydroxyproline

More recently,osteoprotegrin and RANK Ligand are also used as markers of bone turnover.



MARKERS OF BONE FORMATION

S.ALKALINE PHOSPHATASE [71]

S.Alkaline phosphatase, an enzyme located in the membranes of osteoblasts and it is released into circulation during bone formation. In the serum the enzyme exists in various isoforms derived from liver, intestine and placenta, in addition to bone. Hence total Alkaline phosphatase lacks sensitivity and specificity as a bone marker. Bone isoforms exists in three fractions-B/I (70% bone &30% intestine), B_1, and B_2 forms. B_1 and B_2 forms are identified by high performance liquid chromatography. Direct immunoassays using antibodies recogonize the bone

isoenzymes and is found to be more sensitive to detect bone turnover following menopause.

OSTEOCALCIN

Mature osteoblasts,odontoblasts and hypertrophic chondrocytes secretes Osteocalcin ,a small noncollagenous protein containing 49 aminoacids. It is incorporated in to the extracellular matrix of the bone, but a fraction of newly synthesised osteocalcin is released into circulation. Osteocalcin level in serum follows a circadian rhythm characterised by a decline during morning, low around noon and gradually increased during evening and night. Osteocalcin is used as a clinical index of bone turnover because of its tissue specificity ,wide availability and low intra individual variation. But the peptide is rapidly degraded in the serum, resulting in intact and fragmented segments in serum. This resulting heterogenicity of fragments limits its usage as a marker in serum.[44]

PROCOLLAGEN TYPE 1 PEPTIDE

Procollagen type1 contains N and C terminal extension which are removed during extracellular processing of type1 collagen into aminoterminal(P1NP) and carboxyterminal(P1CP) extension peptides before fibril formation. P1NP is useful for monitoring the efficacy of anabolic treatment including parathyroid hormones.

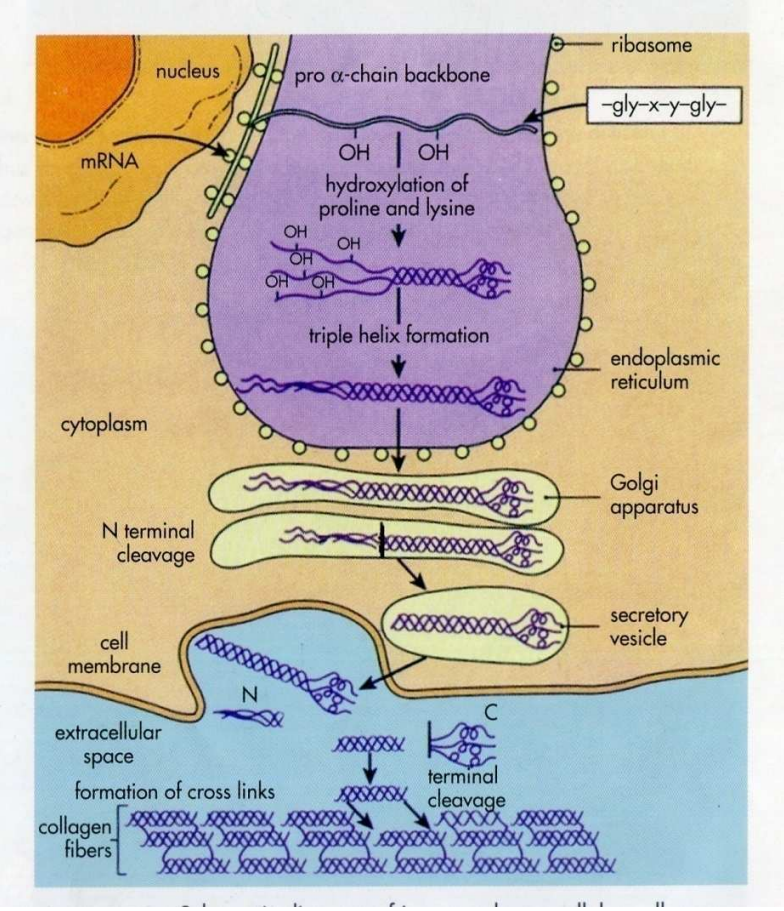
Other protein used as boneturnover markers include osteonectin and bone sialoprotein .

BONE RESORPTION MARKERS

N-TELOPEPTIDE

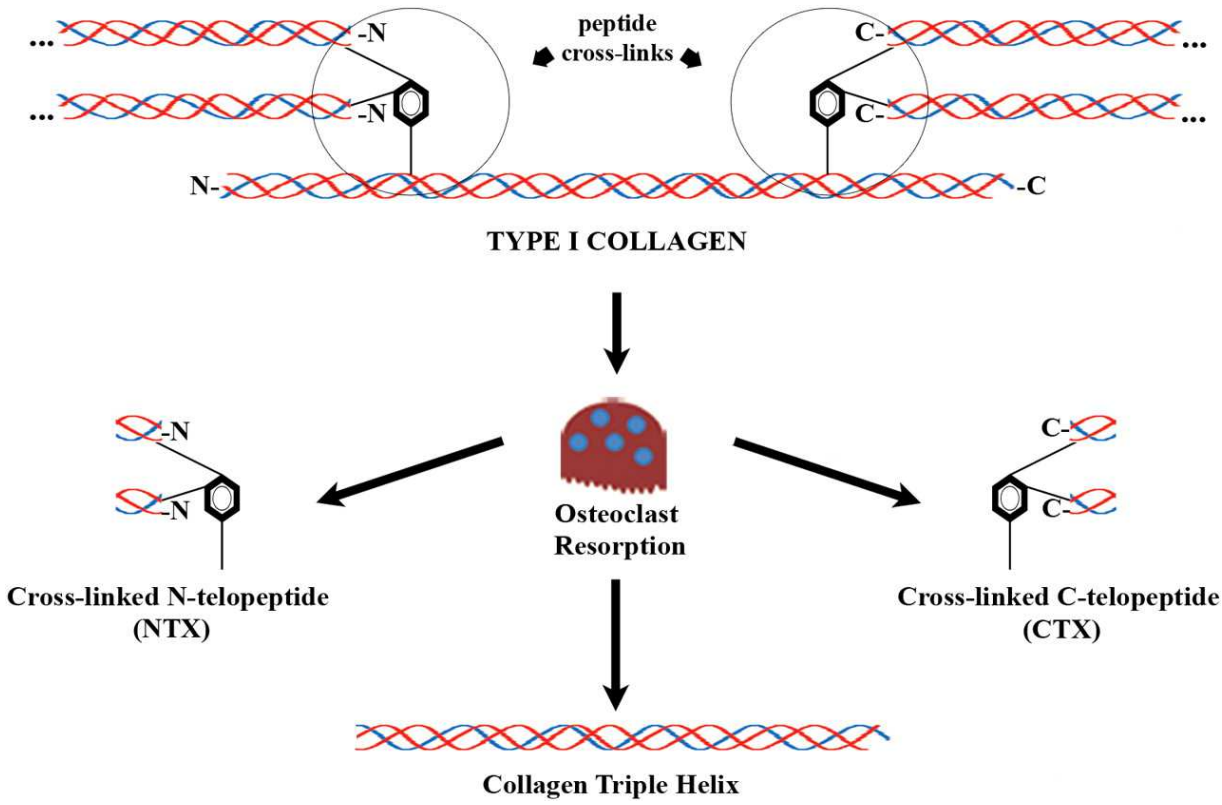
Type 1 Collagen accounts for 90% of organic matrix of the bone. Collagen has triple helix structure with two α_1(I) chains and one α_2 (I) chains. Initially it is synthesised as procollagen with N and C terminal extensions. Procollagen undergoes extensive post translational modifications including hydroxylations of proline and lysine residues, glycosylation and formation of interchain and intrachain disulphide bonds. The N and C terminal extensions are removed by peptidases resulting in the formation of triple helix. Type 1 Collagen immediately assembles into immature fibrils with limited tensile strength andfinally forms mature Collagen by intra and inter molecular cross links.[72]

During the process of resorption amino and carboxy terminal fragments are released. These fragments with attached cross links are called Telopeptides. Elevated levels of N-Telopeptide is seen in postmenopausal women due to increased bone resorption. N-Telopeptide is highly specific for bone because DPD cross links primarily involves α_2 (I) chains with 2/3rd in the N-Telopeptide region.



PYRIDINIUM CROSS LINKS

Extracellular maturation of Collagen produces pyridinium cross links and they are released during bone resorption. Total, free and protein bound PYD and DPD can be measured as bone resorption markers.



TARTRATE RESISTANT ACID PHOSPHATASE

Acid phosphatase is the lysosomal enzymes present in bone, prostate, platelet, erythrocytes and spleen. Bone acid phosphatase is resistant to tartrate and during bone resorption osteoclast produce and release acid phosphatase (isoform 5b) into circulation.[9]

HYDROXYPROLINE

Hydroxyproline is found in Collagen following post translational modification of proline residues and is released into circulation following acid hydrolysis. It is not specific for bone because other tissues like muscle and skin

contains significant amount of collagen. Approximately 10% of the total production is excreted in the urine as the peptide bound form. [73]

The present study is planned to evaluate the levels of N-Telopeptide in postmenopausal women.

AIM AND OBJECTIVES

The aim of the study is to measure S.N-Telopeptide , a bone resorptive biomarker in postmenopausal women and to compare it with healthy premenopausal women.

The objectives of this study are

(I) To correlate Serum N-Telopeptide with Serum Estrogen level.

(II) To correlate Serum N-Telopeptide level with Serum Calcium, Serum Phosphorus and Serum Total Alkaline phosphatase.

MATERIALS AND METHODS

The study was conducted at Thanjavur Medical College Hospital after getting approval from the ethical committee. Written informed consent was obtained from the participants.

STUDY GROUP

Study group included 50 postmenopausal women aged 50-70 years who were not previously diagnosed as osteoporosis and who had BMD T-score $\geq$ - 2.5 and T-score between -1.0 to -2.5.

EXCLUSION CRITERIA

- Diabetes mellitus
- Kidney diseases
- Drugs -steroid

CONTROL GROUP

Control group included 50 premenopausal women aged 25-35 years without any specific illness.

SAMPLE COLLECTION

Under aseptic conditions, 5ml of venous blood was collected from each subject. The vacutainers containing the blood samples were kept at room temperature for 30 minutes and then centrifuged at 2000g for 15 minutes for clear separation of serum. The following parameters were estimated immediately after the serum was separated.

1. Calcium

2. Phosphorus

3. Total Alkaline phosphatase

4. Urea

5. Creatinine

6. Glucose

The remaining aliquot of serum was stored at -20° C in the deep freezer for estimation of serum N –Telopeptide and serum Estrogen.

BMD was assessed by ultrasonography taken in heel and the T-Scores were recorded.

ESTIMATION OF S.N-TELOPEPTIDE

PRINCIPLE OF THE ASSAY

The microtiter plate provided in the kit has been pre coated with an antibody specific to N-Telopeptide. Standards or samples are then added to the appropriate microtiter plate wells with the biotin –conjugated preparation specific for N-Telopeptide and avidin conjugated to Horseradish peroxidase (HRP) is added to each microtiter plate well and incubated. Then a TMB (3,3',5,5' tetra methyl benzidine) substrate solution is added to each well. Only those wells that contain NTX, biotin-conjugated antibody and enzyme –conjugated avidin will exhibit a change in colour. The enzyme-substrate reaction is terminated by the addition of the sulphuric acid solution and the colour change is measured spectrophotometrically at a wavelength of 450 nm ± 2nm.The concentration of N-Telopeptide in the samples is then determined by comparing the O.D. of the samples to the standard curve.

MATERIALS PROVIDED

REAGENT	QUANTITY
Assay plate	1
Standard	2
Sample diluents	20 ml
Biotin -Antibody Diluent	10 ml
HRP-avidin Diluent	10 ml
Biotin Antibody	120 µl
HRP-avidin	120 µl
Wash Buffer	20 ml
TMB substrate	10 ml
Stop solution	10 ml

REAGENT PREPARATION

1. PREPARATION OF WASH BUFFER :

Wash buffer concentrate is brought to room temperature. If crystals are found in the wash buffer concentrate, mix the buffer gently until the crystals dissolve completely. 500ml of wash buffer is prepared by diluting 20ml of wash buffer concentrate into deionised or distill water.

2. PREPARATION OF STANDARD:

The standard vial is centrifuged at 6000 -10,000 rpm for 30 s. A stock solution of 500 nM BCE is prepared by reconstituting the standard vial with 1.0 ml of sample diluent. The standard is allowed to sit for a minimum of 15 mins at room temperature with gentle agitation prior to making serial dilutions. The undiluted standard serves as the high standard (500 nM BCE). The sample Diluent serves as the zero standard (0 nM BCE). Fresh standard is prepared for each assay and used within 4 hours and discarded.

3. BIOTIN-ANTIBODY:

Biotin-Antibody conjugate is centrifuged before opening and diluted to the working concentration using Biotin – antibody Diluent (1:100).

4. HRP-AVIDIN :

The vial is centrifuged before opening and diluted to the working concentration using HRP-avidin Diluent (1:100).

ASSAY PROCEDURE :

Bring all reagents and samples to room temperature before use.

1. 100µl of Standard , Blank, or Sample is added per well. Cover with the adhesive strip. Incubate for 2 hours at 37°C.

2. The liquid of each well is removed.

3. 100µl of Biotin-antibody working solution is added to each well and incubated for 1 hour at 37°C. Biotin-antibody working solution may appear cloudy. Warm up to room temperature and mix gently until solution appears uniform.

3. The liquid from each well is aspirated and subsequently washed. The whole process is repeated three times for a total of three washes.

   Wash: each well is filled with 200 µl of wash buffer and allowed to stand for stand for 2 minutes, then the liquid is removed by flicking the plate over the sink. The remaining drops are removed by patting the plate over the sink. Finally, the remaining liquid drops are removed by patting the plate on a paper towel. For good performance complete removal of liquid at each step is essential.

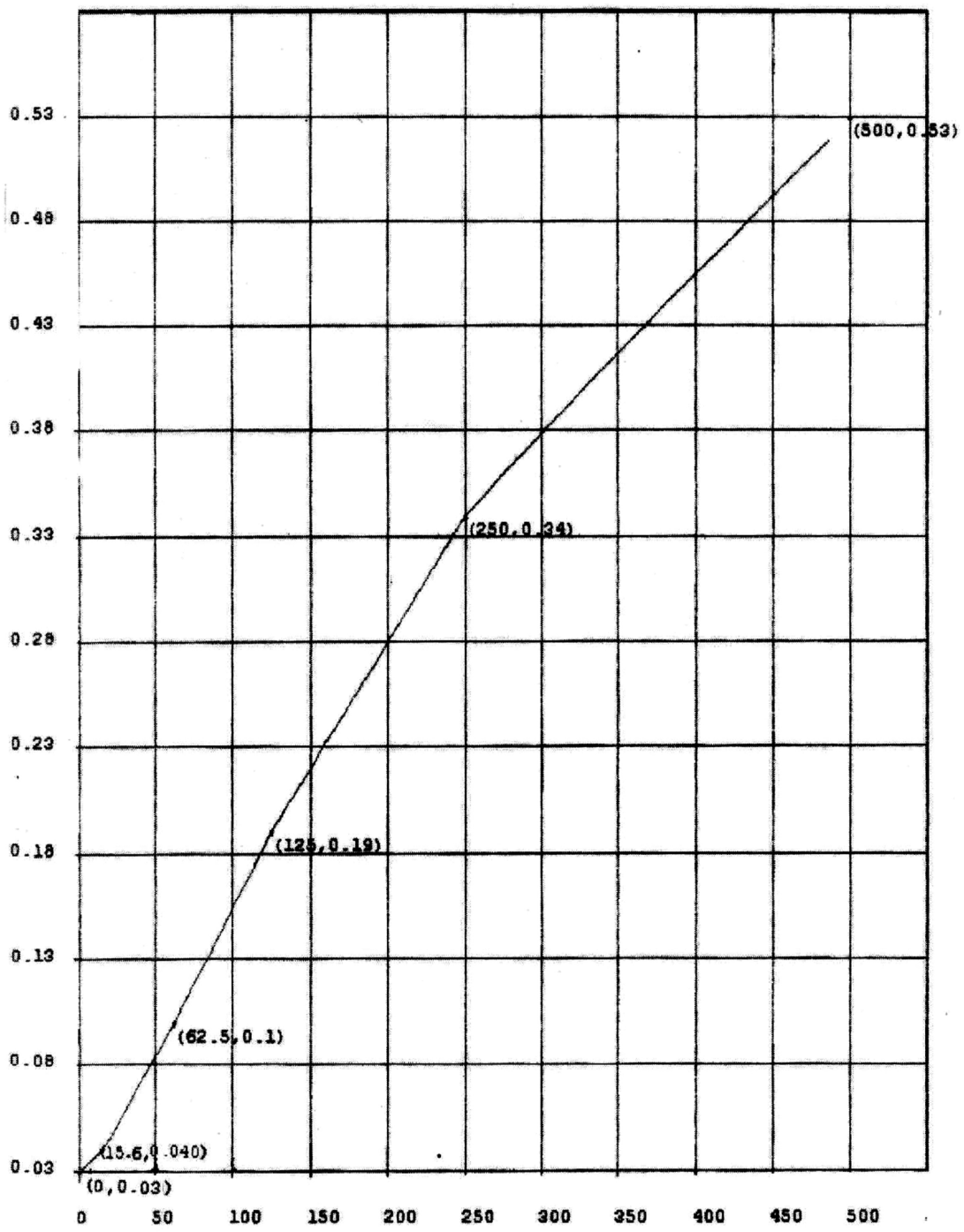
4. 100µl of HRP-Avidin working solution is added to each well. The microtiter plate is then coverd with a new adhesive strip and incubated for 1 hour at 37°C.

5. Aspiration of the wells and subsequent washing is repeated five times as in step 4.

6. 90µl of TMB substrate is added to each well and Incubated for 10-30 minutes at 37°C. The plate is kept away from drafts and other temperature fluctuations in the dark.
7. 50µl of Stop Solution is added to each well when the first four wells containing the highest concentration of standards develop obvious blue colour. If colour change does not appear uniform, gently tap the plate to ensure thorough mixing.
8. The optical density of each well is determined within 30 minutes, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS ;

A standard curve is constructed by plotting the mean absorbance for each standard on the x-axis against the concentration on the y-axis and a best fit curve is drawn through the points on the graph. Using the absorbance value for each sample the corresponding concentration of N-Telopeptide is calculated from the standard curve.


0.53
0.48
0.43
0.38
0.33
0.28
0.23
0.18
0.13
0.08
0.03
0
50
100
150
200
250
300
350
400
450
500
(500,0.53)
(250,0.34)
(125,0.19)
(62.5,0.1)
(15.6,0.040)
(0,0.03)

ESTIMATION OF SERUM ESTRADIOL

Estradiol (E2) is a C18 steroid hormone with a phenolic A ring. It is the most potent natural estrogen produced by the ovary, placenta and in smaller amounts by the adrenal cortex and the male testes.

PRINCIPLE OF THE TEST :

The E2 EIA is based on the principle of competitive binding between E2 in the test specimen and E2 -HRP conjugate for a constant amount of rabbit anti-Estradiol. In the incubation, goat anti-rabbit Ig G-coated wells are incubated with 25 µl E2 standards,controls, patient samples,100µl Estradiol-HRP Conjugate Reagent and 50µl rabbit anti –Estradiol reagent at room temperature (18-25°C) for 90 minutes. During the incubation, a fixed amount of HRP –labelled E2 competes with the endogenous E2 in the standard, sample or quality control serum for a fixed number of binding sites of the specific E2 antibody. Thus, the amount of E2 peroxidase conjugate, immunologically bound to the well progressively decreases as the concentration of E2 in the specimen increases. Unbound E2 peroxidase conjugate is then removed and the wells washed. Next, a solution of TMB Reagent is added and incubated at room temperature for 20 minutes, resulting in the development of blue colour. The colour development is stopped with the addition

of 1N HCL, and the absorbance is measured spectrophotometrically at 450nm. The intensity of the colour formed is proportional to the amount of enzyme present and is inversely related to the amount of unlabelled E2 in the sample. A standard curve is obtained by plotting the concentration of the standard verses the absorbance. The E2 concentration of the specimens and controls run concurrently with the standards can be calculated from the standard curve.

REAGENTS

MATERIALS	**QUANTITY**
Goat Anti-Rabbit IgG – coated microtiter plate	96 wells
Estradiol Reference standards	0.5 ml
Rabbit Anti-Estradiol reagent	7ml
Estradiol-HRP Conjugate reagent	12ml
Estradiol control -1	0.5ml
Estradiol control -2	0.5ml
TMB Reagent	11ml
Stop solution	11ml

REAGENT PREPARATION :

1. All the reagents are brought to room temperature (18-25°C) before use.
2. Samples with expected Estradiol concentrations over 1000 pg/ml may be quantitated by dilution with diluent available from vendor.

ASSAY PROCEDURE:

1. The desired number of coated wells are secured in the holder .
2. 25µl of standards, specimens and controls are added into appropriate wells.
3. 100µl of Estradiol-HRP Conjugate reagent is dispensed into each well.
4. 50µl of Rabbit Anti-Estradiol (E2) reagent is added to each well.
5. Mix it thoroughly for 30 seconds. It is very important to mix them completely.
5. The wells are incubated at room temperature (18-25°C) for 90 minutes.
6. The microwells are rinsed and flicked for 5 times with distilled or de-ionised water.
7. 100µl of TMB Reagent is added to each well and gently mixed for 10 seconds.
8. The wells are incubated at room temperature (18-25°C) for 20 minutes.

9. Finally, the reaction is stopped by adding 100µl of stop solution to each well.
10. Gently mix for 30 seconds till all blue colour changes to yellow colour completely.
11. Absorbance is read at 450nm with the microtiter well reader within 15 minutes.

EXPECTED RANGE:

MALES < 60pg/ml

FEMALES

POSTMENOPAUSAL <18pg/ml

OVULATING PERIOD

EARLY FOLLICULAR	30-100pg/ml
LATE FOLLICULAR	100-400pg/ml
LUTEAL PHASE	60-150pg/ml
PREGNANCY	UPTO 35,000 pg/ml
PREPUBERTAL	<10pg/ml

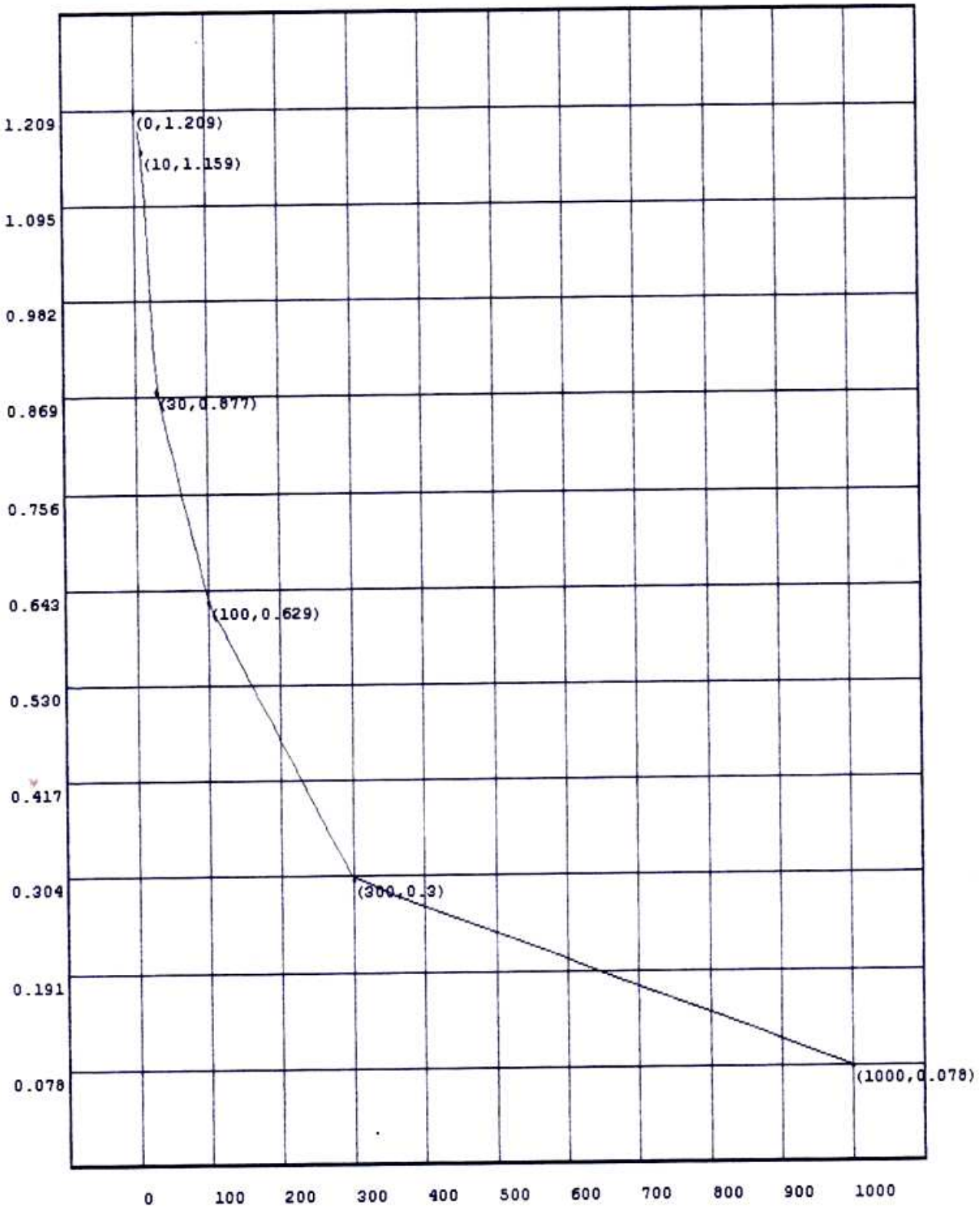
CALCULATION OF RESULTS

1. Calculate the mean absorbance value (A_{450}) for each set of reference standards, controls and samples.

A standard curve is constructed by plotting the mean absorbance obtained for each reference standard against its concentration in pg/ml on a linear-linear graph paper with absorbance values on the vertical or Y-axis and concentrations on the horizontal or X-axis.

The mean absorbance values for each specimen is used to determine the corresponding concentration of Estradiol in pg/ml from the standard curve

The values obtained for diluted samples is further converted by applying the appropriate dilution factor in the calculations.


1.209
1.095
0.982
0.869
0.756
0.643
0.530
0.417
0.304
0.191
0.078
(0,1.209)
(10,1.159)
(30,0.877)
(100,0.629)
(300,0.3)
(1000,0.078)
0
100
200
300
400
500
600
700
800
900
1000

ESTIMATION OF S.CALCIUM

METHODOLOGY: CHEMICAL METHOD -ARSENAZO

PRINCIPLE:

Arsenazo III is chemically stable and has high affinity for Calcium in a neutral pH range. Arenazo forms a 1:1 violet Arsenazo : Calcium complex with an absorbance maximum at 650 nm. The concentration of Calcium is proportional to the absorbance of violet coloured Arsenazo III: Calcium complex. The colour produced by this complex is stable for at least 8 hours at room temperature.

REAGENT COMPOSITION:

REACTIVE INGREDIENTS:

Arsenazo III 233µmol / L

Non reactive buffers, stabilizers and fillers.

TEST PROCEDURE:

Wavelength : 650nm

Into a series of matched cuvettes pipette:

TEST 1ml reagent + 25 µl sample

STANDARD 1ml reagent + 25 µl standard

BLANK 1ml reagent + 25 µl water

Mix and incubate at room temperature. The reaction is complete within one minute. The colour is stable for at least 8 hours. Read the absorbance of the sample and standard at 650 nm, adjusting the instrument to zero absorbance with the blank.

CALCULATIONS:

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \text{ x concentration of standard =mg/dl calcium}$$

LINEARITY:

The assay is linear upto 15 mg/dl.

REFERENCE RANGE:

8.5-10.5 mg/dl.

ESTIMATION OF S.PHOSPHORUS

PRINCIPLE:

Phosphate reacts with ammonium molybdate in acid medium to form phosphomolybdate complex with yellow colour. The intensity of colour is directly proportional to the inorganic phosphate in the sample, the colour formed is measured at 340 nm.

Inorganic phosphorus + Ammonium molybdate → Phosphomolybdate

INTERFERING SUBSTANCES

Hemolysed serum will interfere with the assay.

Glasswares contaminated with detergents will affect the results.

REAGENTS:

REAGENT NO.	REAGENT	COMPOSITION	CONCENTRATION
1	Molybdate reagent	Ammonium molybdate surfactant	0.3 mM/ L
2	Sample blank reagent	Sodium chloride preservative	154 mM/ L
3	Phosphorus standard	Potassium dihydrogen phosphate preservative	5mg/dl

PROCEDURE:

	Reagent Blank	Standard	Test
Serum	-	-	10 µL
Reagent 3	-	10 µL	-
Reagent 1	1000 µL	1000 µL	1000 µL

Mix well and incubate at 37°C for 5 mins.

CALCULATION:

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \text{ x concentration of standard } = \text{mg / dl Phosphorus}$$

REFERENCE RANGE:

CHILDREN 4.0 – 7.0 mg/dl

ADULTS 2.5 – 4.5 mg

ESTIMATION OF S. TOTAL ALKALINE PHOSPHATASE

Alkaline phosphatase is present in high concentration in liver, bone, intestine, placenta and certain tumors.

PRINCIPLE:

2-amino-2-methyl -1-propanol + p- nitrophenylophosphate + $H_2O \xrightarrow{ALP}$

4-nitrophenol+ 2- amino -2- methyl-1- propane phosphate

ALP : ALKALINE PHOSPHATASE

METHODOLOGY:

The ALP- AMP method is based on the recommendations of German society of clinical chemistry. This method utilises 4-nitrophenyl phosphate as the substrate. Under optimised conditions ALP present in the sample catalyses the following reaction.

At the pH of the reaction , 4-nitrophenol has a intense yellow colour. The reagent also contains a metal ion buffer system to ensure that optimal concentrations of zinc and magnesium are maintained. The metal ion buffer can also chelate other potentially inhibitory ions which may be present. The reaction is monitored by measuring the rate of increase in absorbance at 415 nm which is proportional to the activity of ALP in the serum.

REAGENT COMPOSITION

ACTIVE REAGENTS	CONCENTRATION
AMP buffer pH 9.8	350 mmol/l
Mg^{2+}	2.0mmol/l
Zn^{2+}	1.0mmol/l
EDTA	2.0mmol/l
p-nitrophenyl phosphate	16.0mmol/l

WORKING REAGENT PREPARATION:

Mix 4 parts of R1 with 1 part of R2. Reagents are stable when stored between 2-8° C.

ASSAY PARAMETERS:

Mode	Kinetic
Wavelength	405
Woking reagent volume	1000µl
Lag time (secs)	60
No. of readings	2

Kinetic factor	2764
Reaction temperature	37°
Reaction direction	increasing
Normal low	28
Normal high	111
Linearity low	0
Linearity high	1200
Blank	water
Units	IU/L

ASSAY PROCEDURE

Pipette	Sample
Working reagent	1000µl
Sample	20µl

Mix well and aspirate

CALCULATION:

ALP activity (IU/L) = ΔA/min X Factor (2764).

EXPECTED VALUES:

FEMALES	20-50Y	28 – 78 U/L
	≥60	40 – 111 U/L
MALES	20-50Y	38 – 94 U/L
	≥60Y	43 – 88 U/L

ESTIMATION OF BLOOD UREA

METHODOLOGY : UREASE METHOD

Urea is synthesised in the liver as a by product of deamination of amino acids.

PRINCIPLE :

The enzyme urease catalyses the hydrolyses of urea into ammonia and carbondioxide. In the next reaction glutamate dehydrogenase catalyses the conversion of ammonia and α-ketoglutarate to glutamate and water with simultaneous oxidation of reduced nicotinamide adenine dinucleotide (NADH) to nicotinamide adenine dinucleotide (NAD). For one mole of urea two moles of NADH are oxidised.

Urease

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_4^+ + CO_2$$

Glutamate dehydrogenase

$$NH_4^+ + \text{NADH} + H^+ + \text{2-Oxoglutarate} \longrightarrow \text{Glutamate} + NAD^+$$

The initial rate of decrease in absorbance at 340nm is proportional to urea concentration in the sample.

REAGENT COMPONENT AND CONCENTRATION:

R_1 Reagent:

A-Ketoglutaric acid	-	99.8 mmol/L
Urease	-	23.5 KU/L
Glutamate dehydrogenase	-	3.5KU/L
Adenosine Diphosphate	-	7.6 mmol/L
Sodium azide	-	0.2%

R_2 Reagent:

NADH - 2.95 mmol/L

Sodium azide - 0.1%

REAGENT PREPERATION AND STORAGE:

R1 & R2 Reagents are ready to use. Mix 4 volumes of reagent one with 1 part of reagent two. Reagents are stable when stored at 2-8° C and protected from direct sunlight.

PROCEDURE:

	BLANK	**STANDARD**	**TEST**
REAGENT	1.0ml	1.0ml	1.0m1
STANDARD	-	10µl	-
SAMPLE	-	-	10µl

CALCULATION:

ΔA Sample/ΔA standard X 50 = mg/dl urea in the sample

REFERENCE INTERVAL: 15-39mg/dl.

ESTIMATION OF S.CREATININE

METHODOLOGY : MODIFIED JAFFE' S REACTION

PRINCIPLE :

Creatinine reacts with alkaline picrate to produce an orange –yellow colour. Specificity of the assay has been improved by the introduction of initial rate method. The intensity of the orange-yellow colour formed is directly proportional to the creatinine concentration and is measured photometrically at 500-520 nm.

REAGENT COMPOSITION:

REAGENT : 1 Picric acid reagent

Picric acid -25.8 mmol/L

REAGENT : 2 sodium hydroxide reagent

Sodium hydroxide -95 mmol/L

Creatinine standard -2mg/dl

REAGENT PREPARATION:

Mix equal volumes of reagent 1 and reagent 2.

Wait for 15 minutes before use.

ASSAY PROCEDURE:

Pipette	Standard	Test
Working reagent	1000µl	1000µl
Standard	100µl	-
Test	-	100µl

Mix well and read initial absorbance (A_1) 20 seconds after mixing and final absorbance (A_2) 80 seconds after mixing.

CALCULATION :

$\Delta A = A_2 - A_1$

$$\text{Creatinine (mg/dl)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{concentration of standard}$$

LINEARITY : This assay is linear up to 20 mg /dl.

NORMAL VALUES:

MALES : 0.7-1.4 mg/dl.

FEMALES : 0.6-1.2 mg/dl.

ESTIMATION OF BLOOD GLUCOSE

METHODOLOGY : GLUCOSE OXIDASE PEROXIDASE, END POINT / FIXED TIME

PRINCIPLE:

Glucose is estimated by enzymatic method where glucose is oxidised to gluconic acid and hydrogen peroxide in the presence of glucose oxidase. The hydrogen peroxide reacts with phenol and 4- aminophenazone in the presence of peroxidase to form a red violet quinonemine dye as indicator.

REAGENT COMPONENT AND COMPOSITION:

REAGENT 1: Enzyme reagent –Phophate buffer 50 mmol/L, phenol 15mmol/L, 4AAP 2.5mmol/L, GOD 18KU/L, POD 2.5KU/L.

Glucose standard : 100mg /dl (5.54 mmol/L).

PROCEDURE:

	BLANK	STANDARD	SAMPLE
Sample	-	-	10 µl
Standard	-	10 µl	-
Reagent	1000 µl	1000 µl	1000 µl

Mix and incubate for 5 min at 37°C or 15 min.at R.T. measure absorbance of sample (AT) and standard (AS) against reagent blank at 505 nm.

CALCULATION:

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \text{ x concentration of standard = Glucose (mg/dl)}$$

REFERENCE INTERVAL: 60-110 mg/dl (3.33-6.1 mmol/L)

MASTER CHART - I

CONTROL GROUP

S.NO	AGE	HT	WT	BMI	N-TELO	ESTROGEN	BMD	RBG	UREA	CREAT	CALCIUM	PHOS	CaXP	ALP
1	36	1.65	65	23.87511	15.63	128	-0.9	97	22	O.7	10.3	3.8	39.14	56
2	35	1.68	55	19.48696	19	164	-1.1	81	26	0.8	9.7	4.2	40.74	62
3	34	1.58	45	18.02596	16.02	88	-0.8	78	18	0.7	10.5	3.7	38.85	48
4	34	1.57	65	26.37024	10.44	146	-0.5	80	24	0.84	10.2	4	40.8	64
5	31	1.5	58	25.77778	14.28	138	-0.7	76	26	1.02	9.3	3.2	29.76	58
6	32	1.55	52	21.64412	18.98	72	-1	99	21	0.7	10.9	3.7	40.33	68
7	34	1.48	55	25.10957	16.72	212	-0.9	120	24	0.89	10	4	40	85
8	30	1.5	42	18.66667	19.23	136	-1	112	20	0.7	9.3	4.2	39.06	75
9	36	1.58	56	22.4323	19.14	168	-1	98	24	1	9.4	3.8	35.72	53
10	28	1.53	58	24.7768	14.78	170	-0.7	96	21	1.1	9.5	4.2	39.9	58
11	29	1.45	50	23.78121	12.14	98	-0.6	97	26	0.8	10.4	3.6	37.44	74
12	31	1.48	45	20.54419	19.3	106	-1	99	24	0.9	9	4.24	38.16	76
13	35	1.45	38	18.07372	19.28	126	-1	104	28	1.1	10.2	2.9	29.58	82
14	27	1.55	46	19.14672	18.76	132	-0.9	94	26	0.82	9.9	3.8	37.62	56
15	30	1.58	54	21.63115	19.42	144	-1.1	90	24	0.8	10.6	2.9	30.74	48
16	32	1.5	53	23.55556	14.36	188	-0.7	85	28	0.9	10.4	3	31.2	47
17	28	1.6	55	21.48438	16.14	196	-0.8	98	20	0.8	10	3.2	32	65
18	34	1.55	68	28.30385	18.02	78	-0.9	96	30	1	9.4	4.1	38.54	70
19	31	1.56	62	25.47666	19.24	121	-1.1	93	27	0.8	9.6	3.4	32.64	62
20	34	1.54	48	20.2395	17.12	94	-0.8	108	20	1.2	9.9	3.8	37.62	76
21	30	1.6	50	19.53125	11.68	139	-0.6	92	28	0.9	10.2	3.2	32.64	82
22	36	1.52	65	28.13366	19.4	158	-1	109	26	0.77	9	4.1	36.9	72
23	32	1.55	57	23.72529	18.43	165	-0.9	82	24	0.9	9.3	3.9	36.27	60
24	29	1.52	54	23.37258	14.16	117	-0.7	84	20	0.89	9.2	4.3	39.56	89
25	28	1.6	59	23.04688	12.02	106	-0.6	90	18	0.7	10.8	2.8	30.24	91

26	32	1.65	65	23.87511	19.87	74	-1.1	86	24	0.9	9.5	3	28.5	62
27	34	1.46	55	25.80221	15.01	122	-0.7	98	20	0.72	10.4	2.8	29.12	80
28	31	1.55	60	24.97399	15.62	98	-0.8	102	26	0.9	9.9	3.4	33.66	74
29	28	1.6	58	22.65625	12.34	130	-0.6	94	32	1	9.4	4.1	38.54	91
30	30	1.58	60	24.03461	14.82	104	-0.7	108	26	0.8	10.2	2.9	29.58	70
31	32	1.62	64	24.38653	15.21	116	-0.8	100	23	0.9	9.8	3.4	33.32	58
32	31	1.6	50	19.53125	14.62	66	-0.7	98	28	1.1	9.5	3.2	30.4	60
33	30	1.45	50	23.78121	19.67	104	-0.7	95	22	0.8	9	4	36	58
34	34	1.5	55	24.44444	25.55	76	-1	86	21	0.7	9.1	3.8	34.58	64
35	32	1.58	52	20.83	17.6	142	-0.9	87	24	0.9	10.2	2.9	29.58	64
36	28	1.56	54	22.18935	19.3	180	-0.8	82	27	0.8	10.5	2.8	29.4	65
37	30	1.55	50	20.81165	17.14	136	-0.9	98	19	0.6	10.3	3	30.9	57
38	34	1.48	51	23.28342	20.11	148	-0.8	94	22	0.9	9.4	3.6	33.84	87
39	35	1.55	53	22.06035	19.98	78	-1.1	90	26	1	9.4	3.4	31.96	64
40	31	1.49	52	23.42237	19.72	114	-1	104	22	1	9	4.1	36.9	69
41	32	1.57	60	24.34176	18.15	86	-1	110	28	0.8	9.1	4.4	40.04	50
42	34	1.58	45	18.02596	16.43	95	0	112	24	0.8	9	4	36	56
43	36	1.65	65	23.87511	24.76	75	-0.8	98	20	0.7	9.1	3.8	34.58	62
44	30	1.58	52	20.83	18.15	172	-0.9	94	22	0.9	9.4	3.8	35.72	48
45	28	1.55	54	22.47659	15.46	142	-0.7	104	20	0.7	9.9	3.9	38.61	64
46	32	1.57	51	20.69049	17.36	122	-0.8	106	26	0.8	9.6	3.4	32.64	58
47	31	1.6	56	21.875	26.51	124	-0.8	110	22	0.8	9.8	3	29.4	74
48	30	1.54	60	25.29938	16.08	116	-0.7	82	26	0.6	10.2	2.9	29.58	76
49	28	1.55	62	25.80645	17.54	143	-0.7	98	21	0.7	10	3.2	32	47
50	33	1.52	61	26.40235	16.83	136	-0.9	94	24	0.6	9.4	3.8	35.72	79

MASTER CHART - II

STUDY GROUP

S.NO	AGE	SEX	HT	Wt	BMI	N-TELO	ESTROG	BMD	RBG	UREA	CREAT	CAL	PHOS	CaXP	ALP
1	55	F	1.6	70	27.344	129.86	10.1	-3.1	95	28	1	9.1	4.5	41	147
2	60	F	1.47	48	22.213	132.62	10.33	-2.8	90	27	0.81	8.8	4.1	36.1	112
3	58	F	1.5	62	27.556	110.42	13.6	-2.7	84	25	0.66	8.6	4.3	37	87
4	60	F	1.5	52	23.111	109.7	14.23	-2.7	102	20	0.72	8.9	4.8	42.7	148
5	65	F	1.45	48	22.83	90.15	16.8	-2.6	105	28	0.88	9.3	4.2	39.1	100
6	60	F	1.45	45	21.403	107.64	14.44	-2.7	105	20	0.7	9.2	3.6	33.1	119
7	60	F	1.5	40	17.778	139.17	10.6	-3.2	108	22	1	9.3	4	37.2	117
8	65	F	1.46	50	23.457	120.83	14.31	-3	87	24	0.97	8.8	4.5	39.6	144
9	59	F	1.52	70	30.298	95.83	10.3	-2.8	106	22	0.75	9	3.9	35.1	135
10	65	F	1.5	58	25.778	93.75	12.9	-3.4	102	23	0.95	8.9	4.3	38.3	76
11	70	F	1.42	40	19.837	105.6	11.3	-2.8	108	18	0.75	8.1	4.1	33.2	112
12	55	F	1.48	46	21.001	90.83	15.56	-2.8	101	21	0.75	9.7	3.5	34	110
13	55	F	1.32	42	24.105	100.69	12.42	-2.8	95	25	0.74	9.1	3.8	34.6	91
14	56	F	1.58	46	18.427	125.83	14.09	-2.9	99	22	0.7	9.9	3.7	36.6	121
15	57	F	1.5	52	23.111	85.42	16	-2.7	95	28	0.8	9	4.2	37.8	84
16	70	F	1.57	48	19.473	93.83	12.4	-3	108	36	1.2	9.2	3.8	35	129
17	55	F	1.54	48	20.24	88.75	15.42	-2.7	102	24	0.54	9	4.1	36.9	68
18	54	F	1.46	70	32.839	70.05	15.6	-2.6	104	28	0.65	10.3	4.5	46.4	76
19	60	F	1.55	47	19.563	113.19	11.42	-3.2	121	38	1.1	9	4.4	39.6	91
20	58	F	1.55	55	22.893	94.52	13.25	-2.8	110	35	1.02	9.7	3.2	31	78
21	65	F	1.5	48	21.333	102.08	11.8	-2.7	114	19	0.76	9.4	4	37.6	136
22	60	F	1.48	45	20.544	97.63	12.8	-2.6	104	23	0.78	10	4	40	144
23	59	F	1.6	59	23.047	103.85	11.63	-2.5	116	21	1.1	9.7	3.6	34.9	114
24	54	F	1.5	58	25.778	85.42	16.45	-2.5	124	32	1.1	9	4.4	39.6	87
25	61	F	1.48	47	21.457	113.32	11.33	-3	111	20	0.84	9	4.6	41.4	109

26	70	F	1.52	67	29	127.37	10.98	-3.3	102	21	0.7	9.6	3.7	35.5	95
27	58	F	1.51	58	25.44	102.08	12.16	-2.6	93	33	1.02	9.1	4.2	38.2	74
28	57	F	1.45	50	23.78	86.67	15.82	-2.5	94	24	0.9	9.2	3.8	35	86
29	56	F	1.55	52	21.64	97.78	12.64	-2.6	103	40	1	10.6	3.2	33.9	70
30	54	F	1.56	68	27.94	99.87	11.97	-2.7	107	23	0.9	10	3.5	35	144
31	59	F	1.5	65	28.89	104.47	12.01	-2.8	122	24	1	9.8	3.4	33.3	145
32	57	F	1.55	58	24.14	98.15	12.48	-2.6	120	25	0.85	9.7	3.8	36.9	76
33	54	F	1.56	70	28.76	107.48	11.49	-2.8	99	17	0.8	9.6	3.8	36.5	136
34	55	F	1.54	56	23.61	98.59	12.63	-2.8	106	20	0.86	9.9	4.4	43.6	98
35	60	F	1.6	54	21.09	89.37	15.86	-2.7	98	19	0.72	9.2	3.7	34	142
36	58	F	1.52	65	28.13	88.58	14.48	-2.7	109	22	0.77	9	4.4	39.6	90
37	57	F	1.55	57	23.73	102.08	11.96	-2.8	82	20	0.83	9.3	3.9	36.3	108
38	55	F	1.58	56	22.43	128.86	10.03	-3	86	20	0.7	9.4	3.2	30.1	87
39	58	F	1.56	55	22.6	113.42	11.43	-2.9	87	27	0.77	9.4	4.1	38.5	85
40	59	F	1.55	52	21.64	134.16	10.43	-3.2	79	28	0.9	9.3	3.8	35.3	123
41	58	F	1.52	50	21.64	102.61	12.01	-2.8	76	22	0.82	9.5	4	38	138
42	56	F	1.6	65	25.39	70.56	14.01	-2.6	82	24	0.78	9.7	3.7	35.9	75
43	62	F	1.55	55	22.89	87.38	11.43	-2.5	78	30	0.92	9.9	4.3	42.6	96
44	68	F	1.54	52	21.93	101.58	10.15	-2.8	90	20	0.79	9	4.1	36.9	74
45	62	F	1.63	61	22.96	128.65	11.04	-3	86	24	0.8	8.6	4.3	37	82
46	55	F	1.53	68	29.05	71.85	17.34	-2.2	84	25	0.86	9.7	3.8	36.9	92
47	57	F	1.54	68	28.67	68.32	18.65	-2.5	90	34	1	8.4	4.2	35.3	78
48	54	F	1.5	69	30.67	62.15	17.87	-1.9	96	18	0.72	9.6	4	38.4	106
49	52	F	1.58	70	28.04	124.31	11.65	-3.1	88	26	0.9	8.2	3.6	29.5	114
50	54	F	1.57	62	25.15	76.32	17.54	-2.3	95	27	0.7	9.4	3.8	35.7	132

RESULTS & STATISTCS

TABLE: 1

GENERAL DESCRIPTIVE STATISTICS OF CONTROL

AND STUDY GROUPS

	Control (n=50)				*Study (n=50)*			
	Min	***Max***	***Mean***	***S.D***	***Min***	***Max***	***Mean***	***S.D***
AGE	27	36	31.58	2.442	52	70	58.82	4.471
HT	1.45	1.68	1.55	.0542	1.32	1.63	1.522	.055
Wt	38	68	55.00	6.58	40	70	55.94	8.915
BMI	18.0	28.30	22.83	2.55	17.7	32.83	24.09	3.47
N-TELO	10.4	26.51	17.35	3.23	62.1	139.17	101.47	18.39
ESTRO	66	212	125.78	34.79	10	19	13.14	2.310
BMD	-1.10	.00	-.8240	.1954	-3.40	-1.90	-2.76	.273
CAL	9.00	10.90	9.76	0.52	8.10	10.60	9.30	.504
PHOS	2.80	4.40	3.57	0.48	3.20	4.80	3.97	.375
CaXP	28.5	40.80	34.72	3.89	29.5	46.35	36.90	3.27
ALP	47	91	66.28	12.0	68	148	105.62	25.0
RBG	76	120	95.76	9.91	76	124	98.96	12.0
UREA	18	32	23.80	3.21	17	40	24.84	5.36
CREAT	.60	1.20	.845	.135	.54	1.20	.8456	.138

TABLE: 2

ASSOCIATION BETWEEN AGE OF THE RESPONDENTS AND THEIR STUDY AND CONTROL GROUP

S.NO	Age	Group		Statistical inference
		Study (n=50)	**Control (n=50)**	
1	Below 30yrs	0	18 (36%)	X^2=100.000 Df=6 .0001 < 0.05 Significant
2	31 to 35yrs	0	31 (62%)	
3	36 to 40yrs	0	1 (2%)	
4	51 to 55yrs	14 (28%)	0	
5	56 to 60yrs	25 (50%)	0	
6	61 to 65yrs	7 (14%)	0	
7	66 to 70yrs	4 (8%)	0	

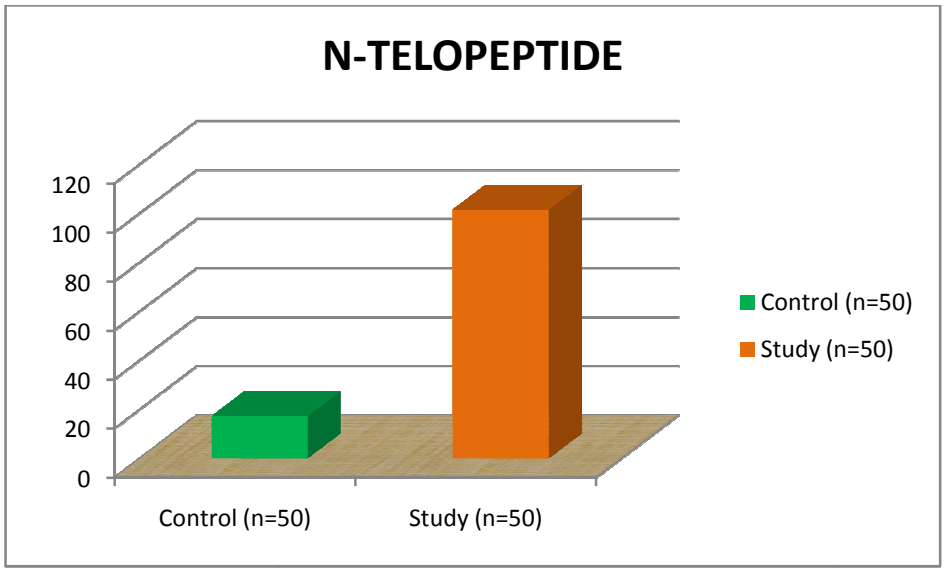
TABLE: 3

STATISTICAL ANALYSIS OF N-TELOPEPTIDE BETWEEN CONTROL AND STUDY GROUPS

T-TEST

S.NO	N-TELO	Mean	S.D	Statistical inference
1	Control (n=50)	17.3504	3.23883	T=-31.840 .0001<0.05 Significant
2	Study (n=50)	101.4728	18.39896	

BAR CHART – 1



COMPARISION OF N-TELOPEPTIDE LEVEL BETWEEN CONTROL AND STUDY GROUP

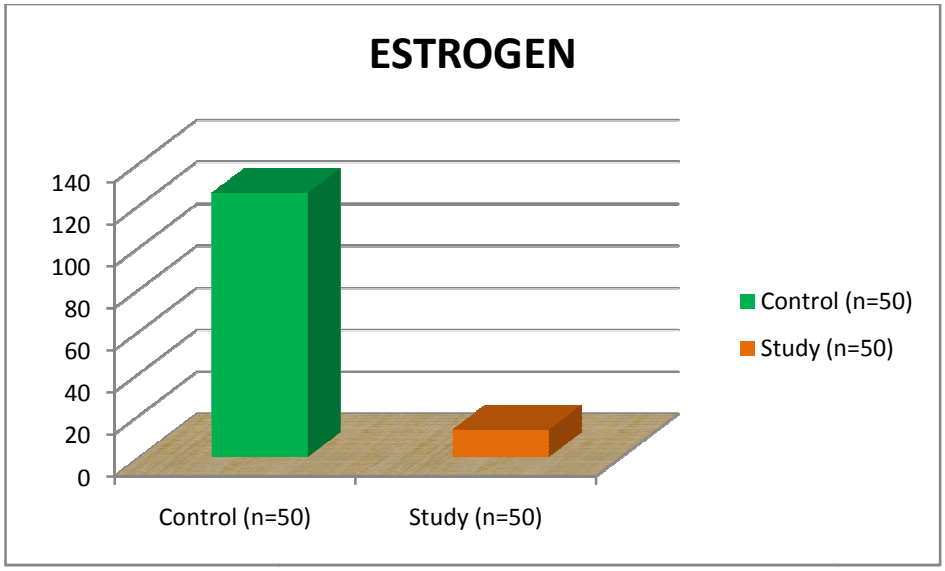
TABLE: 4

STATISTICAL ANALYSIS OF ESTROGEN BETWEEN CONTROL AND STUDY GROUP

T-TEST

S.NO	ESTROG	Mean	S.D	Statistical inference
1	Control (n=50)	125.78	34.793	T=22.841 .0001<0.05 Significant
2	Study (n=50)	13.14	2.310	

BAR CHART -2



COMPARISION OF ESTROGEN LEVEL BETWEEN

CONTROL AND STUDY GROUP

S

TABLE: 5

STUDENT 't' TEST FOR ANALYSIS OF BMD IN CONTROL AND STUDY GROUP

T-TEST

S.NO	BMD	Mean	S.D	Statistical inference
1	Control (n=50)	-.8240	.19542	T=40.827 .0001<0.05 Significant
2	Study (n=50)	-2.7660	.27376	

STUDENT t TEST FOR ANALYSIS OF BMD IN CONTROL AND STUDY GROUP

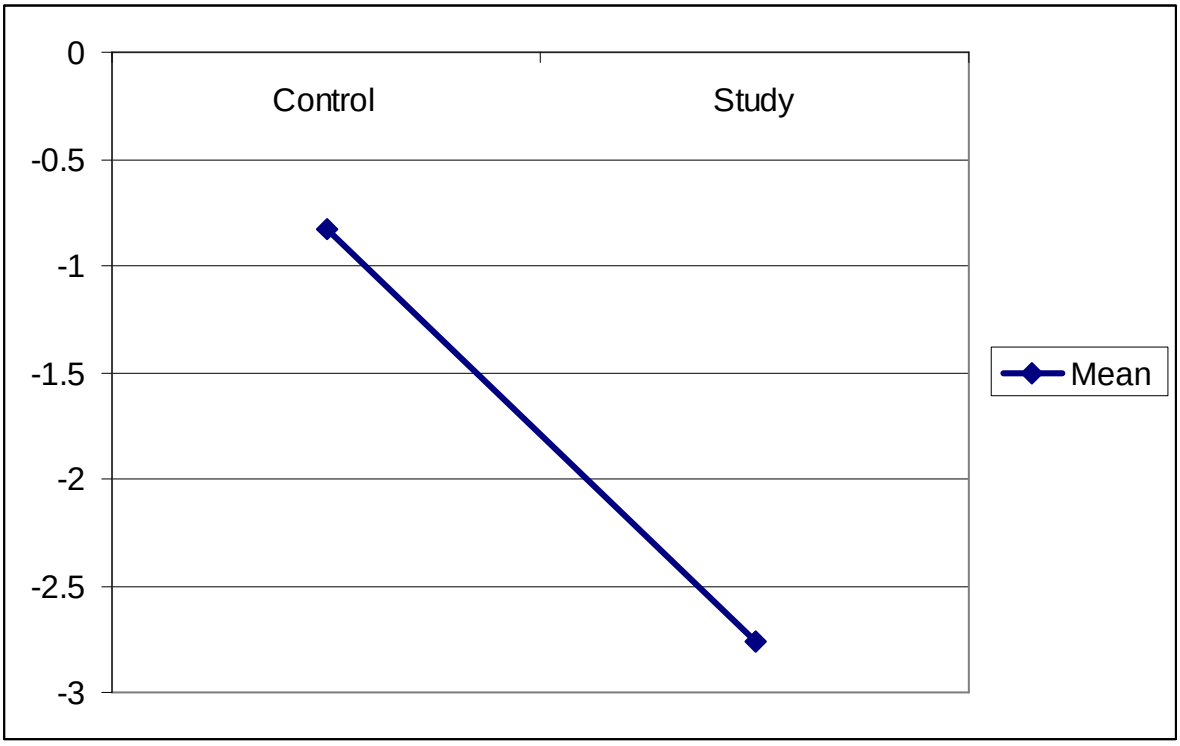


TABLE: 6

STUDENT 't' TEST FOR ANALYSIS OF BMI IN CONTROL AND STUDY GROUP

T-TEST

S.NO	BMI	Mean	S.D	Statistical inference
1	Control (n=50)	22.8383585	2.55797299	T=-2.055 0.043<0.05 Significant
2	Study (n=50)	24.0929245	3.47833764	

BAR CHART – 3

COMPARISION OF BMI BETWEEN CONTROL AND STUDY GROUP

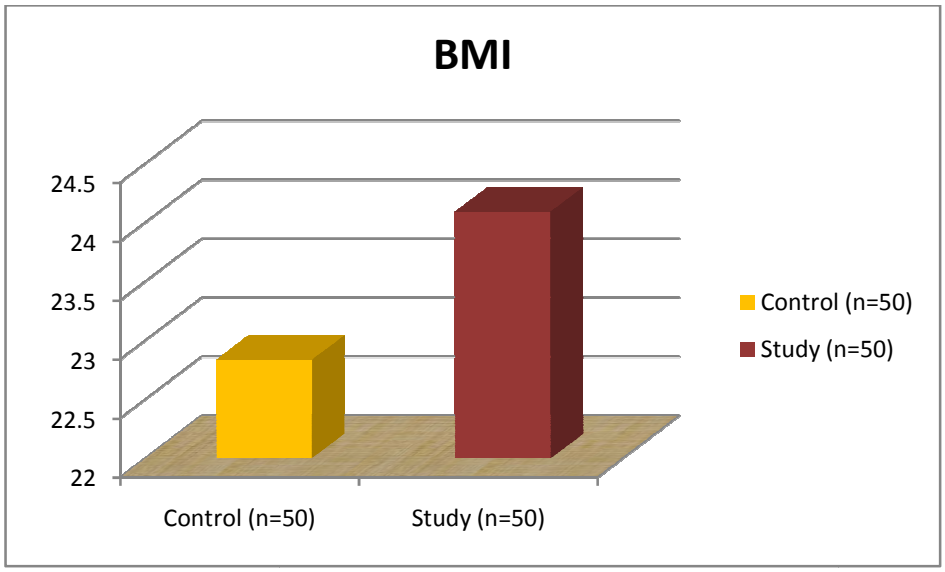


TABLE: 7

STUDENT 't' TEST FOR ANALYSIS OF CALCIUM, PHOSPHORUS AND Ca X P RATIO BETWEEN CONTROL AND STUDY GROUP

S.NO			Mean	S.D	Statistical significance
1	CALCIUM	Control (n=50)	9.7620	0.52911	T=4.451 0.0001 > 0.05 Significant
		Study (n=50)	9.3020	0.50406	
2	PHOSPHORUS	Control (n=50)	3.5728	0.48556	T=-1.176 0.0001 > 0.05 Significant
		Study (n=50)	3.9760	0.37502	
3	Ca X P	Control (n=50)	34.72	3.893	T=-3.040 0.03 > 0.05 Significant
		Study (n=50)	36.90	3.279	

BAR CHART - 4

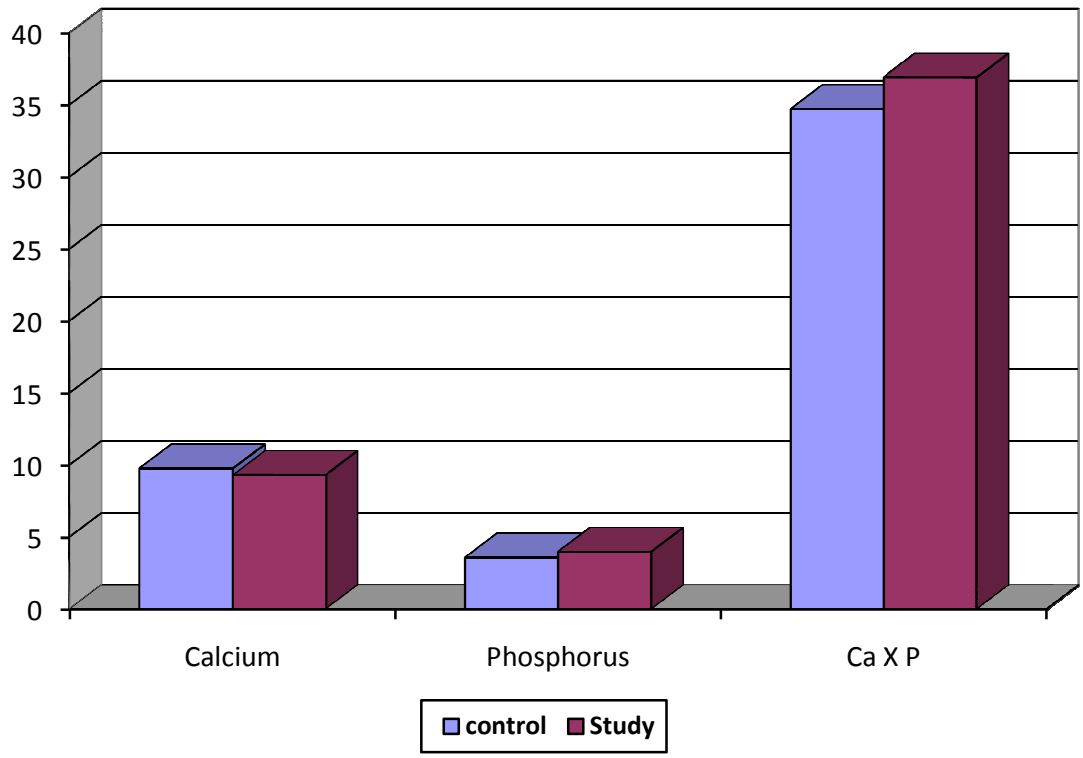


TABLE: 8

STUDENT 't' TEST FOR ANALYSIS OF S. TOTAL ALKALINE PHOSPHATASE IN CONTROL AND STUDY GROUP

T-TEST

S.NO	ALP	Mean	S.D	Statistical inference
1	Control (n=50)	66.28	12.026	T=-10.015 .001<0.05 Significant
2	Study (n=50)	105.62	25.036	

BAR CHART - 5

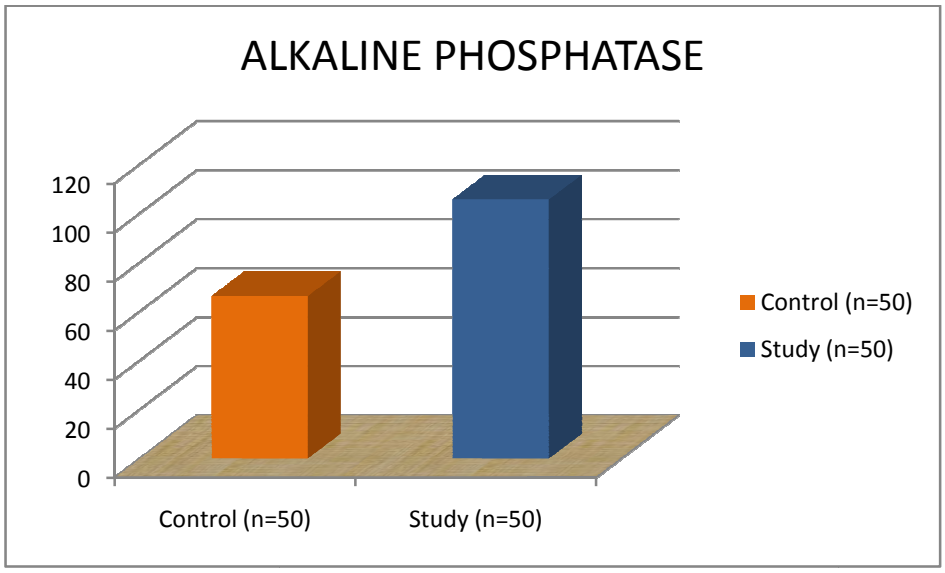


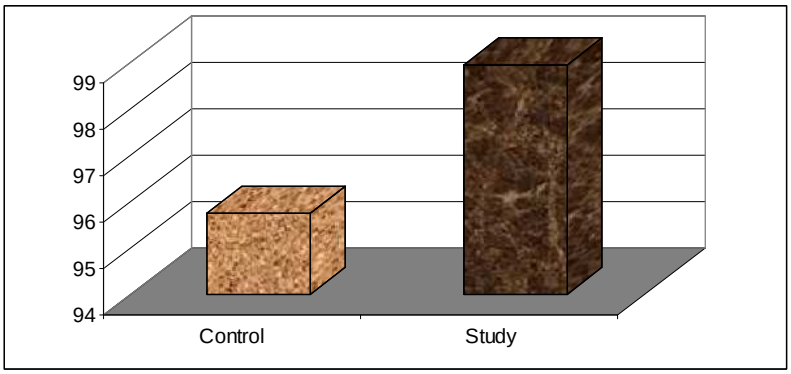
TABLE: 9

STUDENT 't' TEST FOR ANALYSIS OF RBG,UREA, AND CREATININE IN CONTROL AND STUDY GROUP

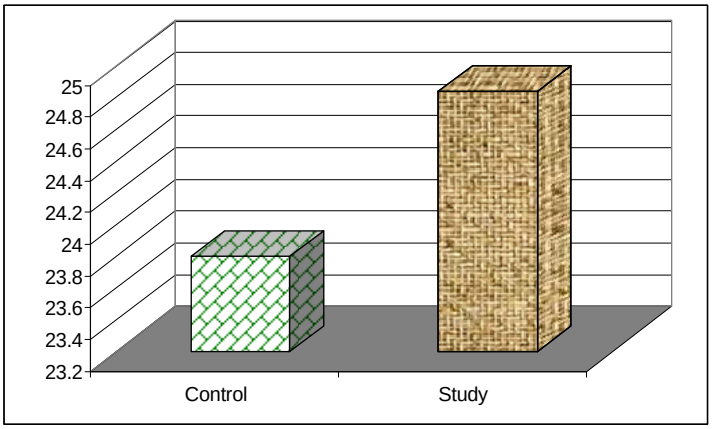
T-TEST

S.NO			Mean	S.D	Statistical significance
1	RBG	Control (n=50)	95.76	9.917	T=-1.453 .149 > 0.05 Not Significant
		Study (n=50)	98.96	12.007	
2	UREA	Control (n=50)	23.80	3.213	T=-1.176 **.**243>0.05 Not Significant
		Study (n=50)	24.84	5.366	
3	CREAT	Control (n=50)	.845	.13573	T=-.022 .983>0.05 Not significant
		Study (n=50)	.8456	.13865	

BAR CHART – 6 BLOOD GLUCOSE



BAR CHART -7 UREA



BAR CHART -8 CREATININE

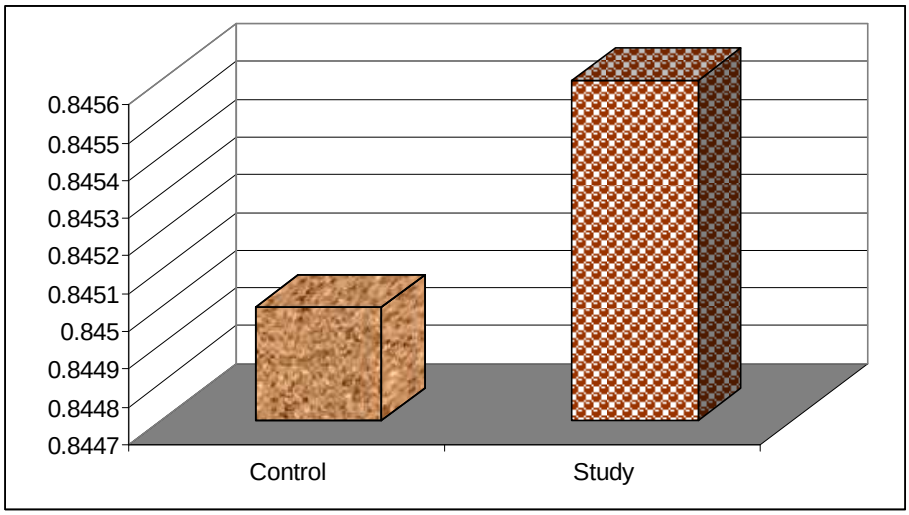


TABLE: 10

ASSOCIATION OF BMD IN CONTROL AND STUDY GROUP

Sl.no	BMD	Group		Statistical inference
		Study (n=50)	Control (n=50)	
1	Less than – 1	0	50 (100%)	X^2=100.000
2	- 1 to -2.5	8 (16%)	0	Df=2
3	> - 2.5	42 (84%)	0	.000<0.05 Significant

TABLE: 11

PEARSONS CORRELATION BETWEEN N-TELOPEPTIDE

AND OTHER STUDY PARAMETERS

Study N-TELO	Correlation value	Statistical inference
BMI	-.372(**)	P < 0.01 Significant
ESTROG	-.745(**)	P < 0.01 Significant
BMD	-.746(**)	P < 0.01 Significant
CAL	-.238	P > 0.05 Not Significant
PHOS	-.015	P > 0.05 Not Significant
Ca XP	-.168	P > 0.05 Not Significant
ALP	.288(*)	P < 0.05 Significant
RBG	-.069	P > 0.05 Not Significant
UREA	-.130	P > 0.05 Not Significant
CREAT	.074	P > 0.05 Not Significant

TABLE: 12

PEARSONS CORRELATION BETWEEN ESTROGEN

AND OTHER STUDY PARAMETERS

ESTROG	**Correlation value**	**Statistical inference**
BMI	.233	P > 0.05 Not Significant
N-TELO	-.745(**)	P < 0.01 Significant
BMD	.648(**)	P < 0.01 Significant
CAL	.039	P > 0.05 Not Significant
PHOS	.088	P > 0.05 Not Significant
CaXP	.118	P > 0.05 Not Significant
ALP	-.169	P > 0.05 Not Significant
RBG	.019	P > 0.05 Not Significant
UREA	.108	P > 0.05 Not Significant
CREAT	-.153	P > 0.05 Not Significant

TABLE: 13

PEARSONS CORRELATION BETWEEN AGE

AND OTHER STUDY PARAMETERS

Study Age	**Correlation value**	**Statistical inference**
BMI	-.344(*)	P < 0.05 Significant
N-TELO	.233	P > 0.05 Not Significant
ESTROG	-.303(*)	P < 0.05 Significant
BMD	-.375(**)	P < 0.01 Significant
CAL	-.278	P > 0.05 Not Significant
PHOS	.191	P > 0.05 Not Significant
CaXP	.028	P > 0.05 Not Significant
ALP	.088	P > 0.05 Not Significant
RBG	.114	P > 0.05 Not Significant
UREA	-.068	P > 0.05 Not Significant
CREAT	.138	P > 0.05 Not Significant

TABLE: 14

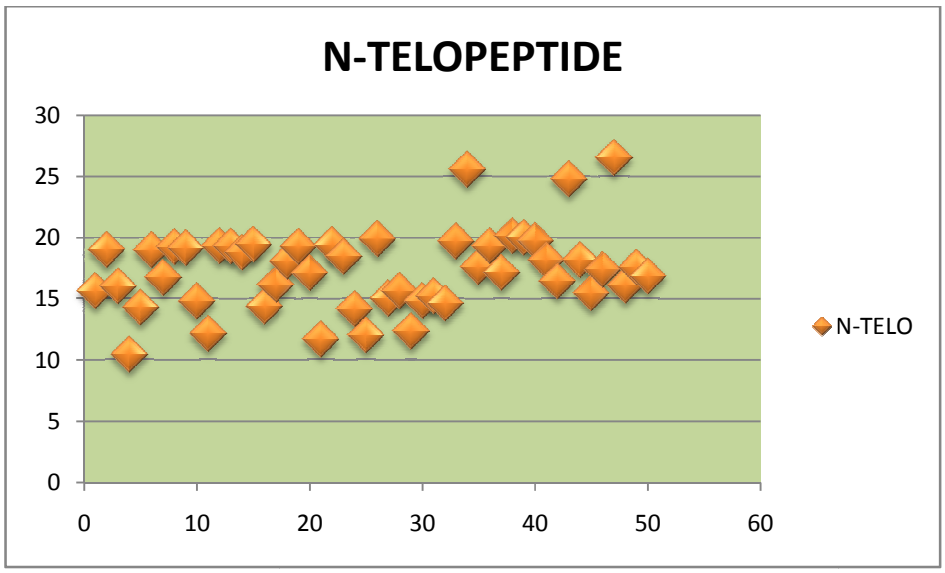
PEARSONS CORRELATION BETWEEN BMD

AND OTHER STUDY PARAMETERS

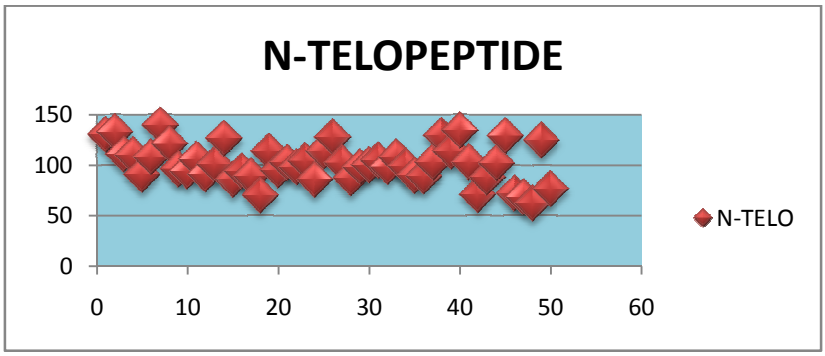
Study BMD	**Correlation value**	**Statistical inference**
BMI	.292(*)	P < 0.05 Significant
N-TELO	-.746(**)	P < 0.01 Significant
ESTROG	.648(**)	P < 0.01 Significant
CAL	.278	P > 0.05 Not Significant
PHOS	-.061	P > 0.05 Not Significant
CaXP	.104	P > 0.05 Not Significant
ALP	-.103	P > 0.05 Not Significant
RBG	-.025	P > 0.05 Not Significant
UREA	-.006	P > 0.05 Not Significant
CREAT	-.165	P > 0.05 Not Significant

SCATTER DIAGRAM

CONTROL GROUP

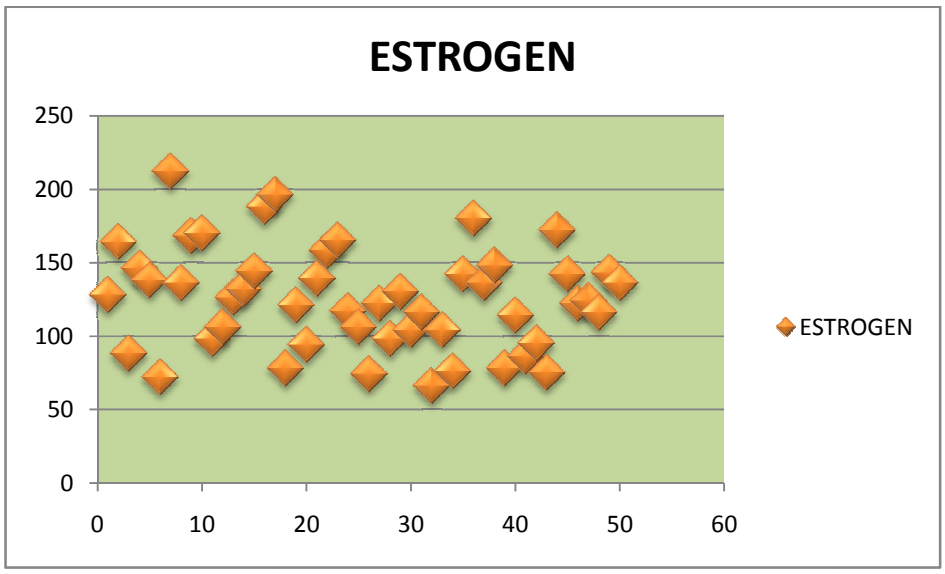


STUDY GROUP

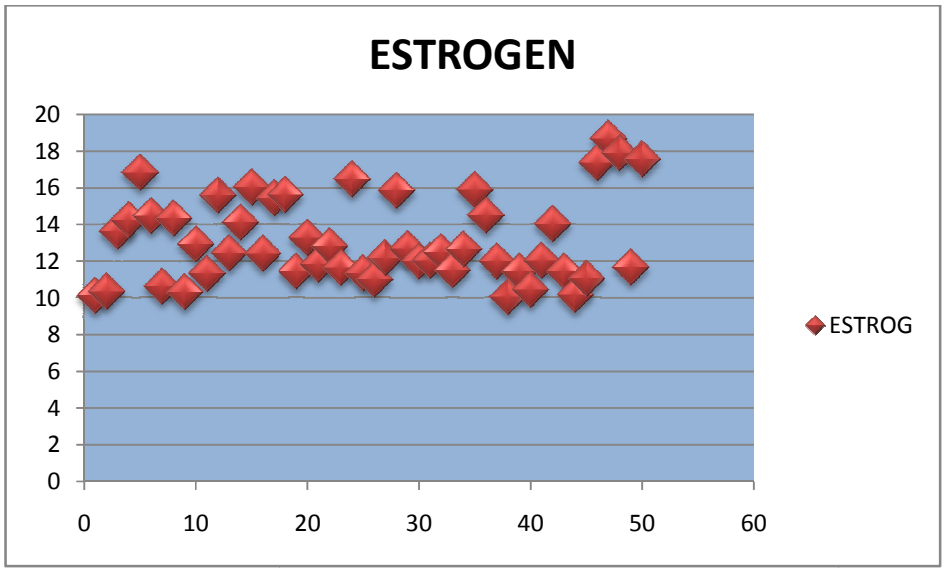


SCATTER DIAGRAM

CONTROL GROUP



STUDY GROUP



RESULTS

A total of 100 subjects are included in the present study. Out of this, 50 are under study group (postmenopausal women with osteoporosis) and 50 are under control group (healthy individuals). The values obtained in the control and study group are presented in the master chart I and II respectively.

The serum values of N-Telopeptide, Estrogen, BMD, Calcium, Phosphorus, Total Alkaline Phosphatase, Random blood glucose, urea and creatinine are estimated in the control and study group. BMI values and ionic product of Calcium and Phosphorus are calculated.

TABLE :1

Shows the anthropometric measurements, the biochemical data, results of N-Telopeptide, Estrogen levels and BMD in control and study group.

In the control group, N-Telopeptide levels are in the range of 10.44 nmol BCE/L to 26.51nmol BCE/L with the mean range of 17.35 nmol BCE/L. In the study group, N-Telopeptide level ranges between 62.15nmol BCE/L to139.17nmolBCE/L with the mean range of 101.47 nmolBCE/L.

In the control group, Estrogen levels are in the range of 66 pg/ml to 212 pg/ml with the mean of 125.78 pg/ml, where as in the study group the range of Estrogen is between 10 pg/ml to 19 pg/ml with the mean range of 13.14 pg/ml.

TABLE: 2

Shows the association between age of the respondents and their study and control group.

TABLE: 3

Shows mean N-telopeptide level between control and study group.

Mean Serum N-Telopeptide in study group is 101.47 ± 18.39 nmolBCE/L which is higher than the control group mean 17.35 ± 3.2 nmolBCE/L which is statistically significant. ($p < 0.0001$)

TABLE: 4

Shows mean S. Estrogen level between control and study group.

Mean serum Estrogen level in study group is 13.14± 2.3 pg/ml which is lower than control group mean serum Estrogen level 125.78 ± 34.79 pg/ml which is statistically significant.($p < 0.0001$)

TABLE: 5

Shows Student 't' test analysis of BMD between control and study group.

This table shows a statistical decrease in the mean BMD level in study group of -2.766 ± 0 .273 when compared to the mean BMD level in control group of -0.8240 ± 0.195 which is statistically significant.

TABLE: 6

Shows Student 't' test analysis of BMI between control and study group.

In this table there is a statistical elevation of mean BMI level in study group of 24.09±3.47 when compared with mean BMI level in control group of 22.83 ± 2.55 which is statistically significant.

TABLE: 7

Shows Student 't' test analysis of S.Calcium, S.Phosphorus and the ionic product of calcium and phosphorus between control and study group.

In this table there is a statistical decrease in mean S.Calcium level in study group of 9.30± 0.504 mg/dl when compared with mean S.Calcium level in control group of 9.76± 0.529 mg/dl which is statistically significant.

Further, there is a statistical increase in mean S.Phosphorus level in study group of 3.976 ± 0.375 mg/dl when compared with mean S.Phosphorus level in control group of 3.5728± 0.4855 mg/dl which is statistically significant.

This table shows mean value of ionic product of calcium and phosphorus in the study group of 36.9±3.27 which is higher when compared to study group of 34.72± 3.89 and it is statistically significant.

TABLE: 8

Shows comparison of mean S.Total Alkaline Phosphatase level between control and study group.

Mean Serum Total Alkaline Phosphatase in study group is 105.62 ± 25.03 U/L which is higher than the control group mean Serum Total Alkaline Phosphatase 66.28 ± 12.02U/L which is statistically significant.

TABLE:9

Shows comparison of mean Random blood sugar, Urea and Creatinine between control and study group.

1. This table shows a statistically insignificant relationship (p value 0.149 > 0.05) between mean random blood glucose levels of study (98.96 ± 12.007mg/dl) and control group(95.76 ± 9.917mg/dl).

2. This table shows a statistically insignificant relationship (p value 0.243 > 0.05) between mean Urea values of study (24.84 ± 5.36 mg/dl) and control group(23.80 ± 3.213 mg/dl).

3. This table shows a statistically insignificant relationship (p:0.983>0.05) between mean Creatinine levels of study (0.8456 ± 0.138mg/dl) and control group(0.845 ± 0.135 mg/dl).

TABLE: 10

Association of BMD between control and study group

This table shows that the control group(100%) is normal and 16% of the study group is osteopenic and the remaining 84% of study group individual is osteoporotic based on BMD which is statistically significant

TABLE: 11

Pearsons correlation between N-Telopeptide and other study parameters.

This table shows negative correlation between N-telopeptide and Estrogen, BMD, BMI and a positive correlation between N-Telopeptide and Total Alkaline Phosphatase, which is statistically significant.

TABLE: 12

Pearsons correlation between Estrogen and other study parameters.

This table shows negative correlation between Estrogen and N-Telopeptide and a positive correlation between Estrogen and BMD,which is statistically significant.

TABLE: 13

Pearsons correlation between Age and other study parameters.

This table shows negative correlation between Age and Estrogen, BMD and BMI which is statistically significant.

TABLE: 14

Pearsons correlation between BMD and other study parameters.

This table shows positive correlation between BMD and Estrogen, BMI and a negative correlation between BMD and N-Telopeptide which is statistically significant.

DISCUSSION

In the era of increased lifespan and longevity, Osteoporosis is the major public health problem. Osteoporosis is the “silent killer”, the individual is usually asymptomatic while the disease follows a relentless course. Only the tip of the iceberg is noticed and the remaining goes for devastating complications like fractures.

The Gold standard in the diagnosis of osteoporosis is the evaluation of BMD by DEXA scan which is not only expensive but also rare in availability. The traditional markers of bone resorption like urinary hydroxy proline and calcium are not highly specific and sensitive. There is always a need felt to have a reliable, non invasive, cost effective, easy accessible and readily available investigation marker to diagnose osteoporosis.

In the present study with 100 subjects, we measured serum N Telopeptide, a bone resorptive biomarker to assess the quality of bone in post menopausal women and compared it with healthy premenopausal women. The strength of the study lies in the fact that both serum N.Telopeptide and BMD measurements were simultaneously assessed in elderly population and in normal young female.

Comparison of mean value of serum N. Telopeptide of the study group (101.47 ± 18.39) with that of control group (17.35 ± 3.23) showed a significant rise

in the study group. This shows that there is increased rate of bone resorption following menopause. This study also correlates with the previous studies done by N. Jayaram et.al.

Further in our study, we observed that the mean Estrogen level in the study group (13.14 ± 2.3) is significantly low when compared to the control group (125.78 ± 34.79) and the p value is statistically significant. This result clearly supports the pathogenesis that postmenopausal osteoporosis is the hormone dependent acceleration of bone loss which occurs after menopause.

The mean BMD in the study group (- 2.766 ± 0.27) is significantly low when compared to that of control group (-0.82 ± 0.195).

The mean age of the study group is 58.8 years and the mean age of the control group is 31.5 years. Pearsons correlation studies showed a positive relationship between age and serum N Telopeptide and a negative relationship between age and other parameters like BMD and Estrogen. These findings supports the fact that the ageing microenvironment also contributes to decreased formation of bone due to decreased proliferating capacity of osteogenic cells. . Since the mean age of the study is 58.8, this correlates with the current scenario that osteoporosis occurs in Indian population 1 to 2 decades earlier than western population.[74]

Within our study group of 50 individuals, 5 individuals have BMI < 20 and their serum N Telopeptide levels were significantly high when compared to other individuals within the study group. This shows that thin built individuals are at high risk of developing osteoporosis than obese individuals. Obese individuals have larger body mass which imposes greater mechanical load on bone thereby increasing the bone mass. Further adipocytes are important source of estrogen in postmenopausal women which inhibits bone resorption by osteoclasts.

In our study S.Calcium, S.phosphorus and S. Total Alkaline phosphatase shows no significant correlation with serum N Telopeptide, which in turn suggests that these parameters do not reflect the same aspects of bone metabolism as that of serum N. Telopeptide.

With regard to S.Calcium the blood level is tightly regulated within normal limits by Parathyroid hormone otherwise, alterations in the homeostasis of S.Calcium may lead on to life threatening complications.

Though there is a significant increase in the mean level of S. Total Alkaline phosphatase in the study group (105.62 ± 25.03) when compared to that of control group (66.28 ±12.02) it cannot be considered as a reliable marker for osteoporosis because it is not specific for bone. The blood level of S.Total Alkaline phosphatase

is contributed by various tissues like liver, placenta, intestine in addition to osteoblasts of bone.

The other parameters like urea, creatinine and random blood glucose does not show any possible relationship with primary postmenopausal osteoporosis.

In the Pearsons correlation analysis the serum N Telopeptide shows significant negative correlation with BMD, Estrogen and BMI and a positive correlation with S.Total Alkaline phosphatase.

These observations finally suggests that the level of S.N.Telopeptide, the cross linked collagen peptides increases with bone resorption and can be used as a reliable marker in primary postmenopausal osteoporosis.

CONCLUSION

This study shows that there is a significant increase in serum N.Telopeptide level in postmenopausal women and it correlates well with the degree of osteoporosis. Further, measurement of serum N.Telopeptide also helps to identify the individuals having high bone resorption rate and hence increased fracture risk. Hence earlier interventional measures both by dietary and pharmacological means can be taken to reduce fracture and its complications.

LIMITATIONS OF THE STUDY

Our study has the following limitations

- Sample size is very small
- Neither PTH nor Vitamin D, the regulators of mineral metabolism are included in the study.

Further, variations that occur due to seasonal changes - increased bone resorption during winter than summer are not considered in the study.

SCOPE FOR THE FUTURE STUDY

Osteoporosis is a complex, multifactorial, polygenic disease in which genetic determinants are modulated by hormonal, nutritional and environmental factors. In postmenopausal osteoporosis, deficiency of estrogen plays a major role in bone loss. Estrogen regulates bone homeostasis by acting through ER α and ER β .These receptors are the members of the nuclear receptor super family which act as ligand activated transcription factors. Different polymorphisms have been reported in ER α and ER β genes. Studies can be done in future to determine the contributions of these polymorphism in the pathogenesis of osteoporosis and also to define the functional molecular consequences in the causation of osteoporotic phenotype.

Pharmacotherapy for osteoporosis has been mainly focused on interventions that could reverse excessive bone resorption. cDNA analysis studies have shown that Cathepsin K, a protease which degrade protein matrix of the bone is expressed at high levels in the contact site between osteoclast and bone resorption pit. This provides a scope for the development of new antiresorptive drugs like Cathepsin K inhibitors in the treatment of osteoporosis.

BIBLIOGRAPHY

1. Peter G. Bullough. Diseases resulting from disturbances in cell linkage. In: Orthopaedic pathology. 4 th Edition. Mosby publication ; P. 189-190.
2. N.Jayaram, A.R. Bijoor et al., The value of serum and urinary N-Telopeptide in the diagnosis of osteoporosis. Indian Journal of Orthopaedics 2002; 36(2):1-5.
3. AB Goregoankar, Mukesh Phalak, Deepak Joshi .Markers of osteoporosis Current status and role in osteoporosis. Orthopaedics Today vol. XIII NO.1 : Jan-Mar 2011.
4. Andrew E. Rosenberg. Bones, joints and soft tissue tumors.In: Vinay kumar, K.Abbas, Fausto N., Jon C. Aster editors. Robbins and Cotran Pathologic Basis of disease . 8th edition. Philadelphia: Saunders Elsevier; P.1214-1215.
5. Edward F.McCarthy, Frank J.Frassica. Metabolic bone diseases.In: Pathology of Bone and Joint Disorders with clinical and radiological correlation. Philadelphia : W.B.Saunders company ; P .91.
6. Vincent J. Vigorita. Metabolic Bone Diseases. Orthopaedic Pathology. 2nd edition.Philadelphia : Wolters Kluwer/Lippincott Williams & Wilkins ; P.112.
7. Richard Eastell, Rosemary A. Hannon: Biomarkers of bone health and osteoporosis risk. Procceedings of the nutrition society (2008) ; 67: 157-162.

8. Yoshifumi Maeno, Masaaki Inaba et al., Serum concentrations of cross linked N-Telopeptides of type I collagen: New marker for bone resorption in hemodialysis patients, Clinical chemistry (2005) 51;12: 2312-2317.

9. Felica Cosman and David dempster. Pathogenesis of osteoporosis. Metabolic bone diseases. Marc C.Hochberg, Alan J.Silman et al.,editors. Rheumatology. 4th edition. Elsevier publication: P.1937-1939.

10. Lawerence G.Raisz. Pathogenesis of osteoporosis: concepts, conflicts and prospects. Journal of clinical investigation .2005; 115(12): 3318-3325.

11. LJ Chaudhary, Rupesh kumar. Osteoporosis: Investigations helping early diagnosis. Orthopaedics today. volume 12; no.1: Jan-Mar 2010.

12.Ego Seeman. Osteoporosis : a public health problem. Roger Detels, James McEwen , Robert Beaglehole,Tanaka H, editors. Oxford Text book of Public Health . 4th edition. Oxford University Press : P. 1299-1300.

13. M.Doherty, P.Lanyon et al., Musculoskeletal disease. Nicki R.Colledge, Brain R. Walker, Stuart H. Ralston.editors. Davidson's Principles and practice of medicine.21st edition. Elsevier :P. 1116-1117.

14. Balu sankaran. Recent developments in the study of bone physiology and osteoporosis. Osteoporosis : Clinical, Radiological, Histological ,Assessment and an Experimental study.P.182.

15. Rajesh Malhotra, Ravijot singh Muhammad V. Mineral transporters –The novel approach in management of bone health and osteoporosis : Orthopaedics Today vol 12 No.1:Jan-Mar 2010.

16.Benjamin L.Hoch, Michael J. Klen, Alan L. Schiller. Bones and joints. Raphel Rubin, David S.Strayer et al., Rubin's Pathology . 5th edition. Philadelphia. Wolters Kluwer/Lippincott Williams & Wilkins Publication: P. 1087.

17. Samuel L.Turek. Histology and histopathology of bone. Orthopaedics principles and their applications.vol I. 4thedition. Lippincott –Raven publication : P. 34.

18. Ersi Voskaridou and Evangelos Terpos. New insights into the pathophysiology and management of osteoporosis in patients with beta thalassaemia, British journal of Haematology. Blackwell publishing Ltd. 2004; 127-139.

19. Wolfgang Sipos, Peter Pietschmann et al., Pathophysiology of osteoporosis. Austria. Springer – Verlag(2009) 159/9-10: 230-234.

20. Moustapha Kassem and Kim Brixen .Pathophysiology, Prevention and treatment of age – related osteoporosis in women. Suresh I.S.Rattan and Moustapha Kassem.editors. Prevention and treatment of age – related osteoporosis in women. 2006 Springer : P. 90.

21. Robert K.Murray. The Extracellular matrix . Robert K.Murray, David A Bende et al., editors. Harper s illustrated biochemistry. 28th edition, Mc Graw Hill publication : P. 539-540.

22. U.H. Lerner. Bone remodeling in postmenopausal osteoporosis. Journal of Dental Research 2006: 85(7): 584-595.

23. F.Feldmen,D.C.Dahlia. Bone and bone tissue. Claus Peter Adler.editor. Bone diseases-macroscopic, histological and radiological diagnosis of structural changes in skeleton . Springer publications : P. 10.

24. Roger M.Atkins, The Musculoskeletal system. Mercer's Orthopaedic surgery. 9 edition. Springer publications : P.70-71.

25. Christopher J., Lauren E. LaMont et al. Sex and gender considerations in male patients with osteoporosis. Clinical orthopaedics and related research, (2011) 469 : 1906-1912.

26. Peggy M.Cawthon. Gender differences in osteoporosis and fractures, Clinical orthopaedics and related research. (2011) 469:1900-1905.

27. SV Huilgol, Naser A Tadvi. Bisphosphanates – newer therapeutic strategies in prevention and treatment of osteoporosis. Orthopaedics Today. vol.XIII No.1 Jan-Mar 2011.

28. Robert Lindsay, Felicia Cosman. Osteoporosis. Anthony S.Fauci, Eugene Braunwald et al. Editors. Harrison's principles of Internal Medicine. 17th edition. Vol. II. McGraw Hill publication: P. 2399-2400.

29. B.Lawrence Riggs. The mechanisms of estrogen regulation of bone resorption. The Journal of clinical investigation. (2000) ; 106: 1203-1204.

30. Clifford Rosen. Osteoporosis. Lee Goldman and Andrew I. Schafer.Editors. Goldman's Cecil Medicine . 24th edition.Philadelphia. Elsevier publications : P. 1578.

31. Sundeep Koshla, L Joseph Melton III et al., The unitary model for estrogen and the pathogenesis of osteoporosis : Is a revision needed ? Journal of bone and mineral research. March 2011; vol.26. no.3 : P. 441-451.

32. K. Henriksen,N.A.Sims et al. The effect of sex steroids on osteoclast function. A.R.Genazzani. Editors. Postmenopausal osteoporosis .Taylor and Francis Group: P.31-33.

33. Stuart H.Ralston. Genetic determinants of bone mass and osteoporotic fractures. John P. Bilezikian, Lawrence G.Raisz et al. Editors. Principles of bone biology. 3rd edition. Vol. 2. Elsevier publication : P.1616-1617.

34. Cytokines, estrogen and postmenopausal osteoporosis-The second decade, Endocrinology. 1998 ; 139(6) : 2659-2661.

35. Steven L. Teitelbaum. Postmenopausal osteoporosis. T Cells and immune dysfunction. PNAS, November 30,2004:101(48) :16711-16712.

36.Ousamme Hani, Reginald C.Tsang. Bone diseases. Lawrence A. Kaplan, Amadeo J.Pesce. Editors. Clinical chemistry. Elsevier publications: P. 628.

37. D.M.Reid. Diagnosis : clinical, hormonal and biochemical evaluation. A.R.Genazzani. Editor. Postmenopausal osteoporosis. Taylor and Francis Group: P.107.

38.Alan L.Schiller. Bones and joints. Emanuel Rubin and John L.Farber.Editors. Pathology. Philadelphia. J.B. Lippincott publication : P. 1342.

39.Louis Solmon. Metabolic and endocrine disorders. Apley ʻs system of orthopaedics and fracture. 9th edition. Arnold publications :P.132-133.

40. N.A.Athanasou. Osteoporosis. Pathological basis of Orthopaedic and Rheumatic Disease. London .Arnold publications : P. 139.

41.Chrisy L Crowther. The Musculoskeletal and integumentary system. Sue E.Huether,Kathryn L.McCance et al., Editors. Understanding Pathophysiology 4th edition. China. Elsevier publication: P. 1036.

42.J.Christopher Gallagher and Prema B.Rapuri. Osteoporosis: Pathophysiology. Helen L.Henry and Anthony W.Norman. Editors. Encyclopedia of hormones. Vol. 3. Academic Press (Elsevier Science) : P.90-95.

43. J.P.Wali, R.Handaand P.Aggarwal. Osteoporosis. Dr.O.P. Sharma. Editor.Geriatric care in India. P.305-306.

44. David B. Endres, Robert K.Rude. Mineral and bone metabolism.In :Carl A. Burtis Edward R.Ashwood et al., editors. Tietz Text book of clinical chemistry and molecular diagnostics. 4th ed. New Delhi: Saunders Elsevier ; 2006. P. 1777.

45. Jan Dequeker. Metabolic Bone Diseases :Osteoporosis. In:John H Klippel, Paul A Dieppe et al. Editors. Rheumatology . Mosby publication page : P.32.7 (7).

46.Nancy Lane, Meryl S. Leboff. Metabolic bone diseases. Edward D. Harris, Ralph C.Budd et al. Editors. Kelly s Textbook of Rheumatology .volume 2. Elsevier publication : P. 1475 -1477.

47.William W.Hard, Lawrence S.Amesse etal., Menopause. Jonathan S.Berek. Editor. Novak ’s Gynecology. Thirteenth edition. Lippincott Williams and Wilkins publication : P. 1122-1123.

48. Robert Lindsay and Felicia Cosman. Pathogenesis of osteoporosis. Rogerio A.Lobo,Treatment of postmenopausal woman . Third edition.Elsevier publication :P. 323-328.

49. Alan Stevens and James Lowe. Metabolic bone diseases. Pathology. second edition . Spain. Mosby publication : P. 513-514.

50. Pooneh Salari. Successess in understanding pathophysiology of osteoporosis. International Journal of Pharmacology 7 (6) : 677-678, 2011.

51. Jennifer L.Kelsy. Musculoskeletal disorders. Robert B.Wallace, Neal Kohatsu et al., Editors. Public Health and Preventive Medicine. fifteenth edition. McGraw Hill publication :P. 1127.

52.R.L.Prince. Bone diseases in the elderly. John A.H.Wass and Stephen M.Shalet. Editors. Oxford Textbook of Endocrinology and Diabetes. Oxford University Press :P. 424.

53. Francis S. Greenspan, David G.Gardner. Mineral metabolism and metabolic bone disease. Basic and clinical endocrinology. 6th edition. Lange Medical Book /Mc Graw Hill : P. 312-313.

54. Marianne Genge Jagmin. Structure and function of musculoskeletal system. Lee-Ellen C.Copstead Jacquelyn L.Banasik. Editors. Pathophysiology Biological and Behavioural Perspectives. 2nd edition. Saunders company :P. 1129.

55. Ego seeman. Modeling and remodelling. John P.Bilezikein, Lawrence G.Raisz et al., Principles of bone biology. III edition. vol.I. Elsevier publication : P.18.

56.John A. Kirkpatrick. Metabolic diseases of bone. Disturbances in normally formed skeleton. Orthopaedic diseases physiology, pathology, radiology. 3rd edition.Philadelphia. W.B. Saunders company:P.421.

57. Susan V.Bukata. Metabolic bone diseases and osteonecrosis. Walter B.Greene. Editor. Netter s Orthopaedics .Elsevier Saunder publication:P.34.

58. Dr.Indhumati V, Patil V S . Biochemical markers of bone remodelling in osteoporosis –Current concepts . Journal of clinical and diagnostic research 2010 Feb vol.4 (1) : 2089-2097.

59.Pandit S N and Khan R .Postmenopausal Osteoporosis. The Journal of General Medicine. July-Sep 2010; 22 (3) : 12.

60. Sukumar Mukherjee. Investigations and Diagnosis of Bone Disorders. Yash Pal Munjal. Editor.API Textbook of Medicine. 9th edition. vol.I. Jaypee publications: P.302.

61. Theresa Guise and Aillen Heras – Herzig. Osteoporosis and Osteopenia. Mark D. Miller, Jennifer A.Hart et al., Editors. Essential Orthopaedics.Saunders Elsevier publication :P.102.

62. Yun Huang, Vijay laxmi Grey et al., Establishment of reference intervals for bone markers in children and adolescents : Clinical biochemistry 44 (2011) 771-778.

63. Garnero P, Delmas PD. Biochemical markers of bone turnover: clinical usefulness in osteoporosis : Ann Biol Clin (Paris) 1999 Mar-Apr, 57(2) : 137-148.

64.Pierre D.Delmas, Harry K.Genant. Bone density and imaging of osteoporosis. J. Larry Jameson, Leslie J. De Groot et al., Editors. Endocrinology Adult and Pediatric .6th edition. vol.I. Saunders Elsevier publication :P.1280-1281.

65. Gamero P, Hausherr et al., Markers of bone resorption predict hip fracture in elderly women : the EPIDOS Prospective study, J Bone Miner Res.1996 Oct ; 11(10) : 1531-8.

66. Susan L. Greenspan, Harold N. Rosen et al: Early changes in S.N-Telopeptide and C-telopeptide cross linked collagen type I predict long term response to alendronate therapy in elderly women. Home/2000 Archive/October 2000:85(10):3537.

67. Apurva K.Srivastava and David J.Baylink. Biochemical Markers of Bone and Cartilage Turn over. Michael Maricic and Oscar S.Gluck.Editors. Bone Disease in Rheumatology . Lippincott Williams &Wilkins publication : P. 27.

68. S.Rajpar, C.Massard et al. Urinary N-Telopeptide is an independent prognostic factor for overall survival in patients with bone metastases from castration resistant prostate cancer. Annals of Oncology 2010 ; 21:1864-1869.

69. Pierre D Delmas, Richard Eastell et al. Effect of monitoring bone turnover markers on persistence with risedronate treatment of Postmenopausal osteoporosis. The Journal of Clinical endocrinology and metabolism 92(4):1296-1304.

70.Stephan Becker. Surgical operations in orthopaedic spine. Sureshan sivananthan,Eugene sherry et al.Editors. Mercer's textbook of Orthopaedics and Trauma. 10th edition, Hodder Arnold publication : P.1027.

71. osteoporosis biomarkers . Alternative Medicine Review.2007 ; 12(2):120-122.

72. Pamela C.Champe,Richard A.Harvey et al., Fibrous protein. Lippincott ‘s illustrated review:Biochemistry.4 thedition. Walters Kluwer /Lippincott publication :P. 43-48.

73. Nik mohd ismal. Post menopausal osteoporosis : epidemiology, pathophysiology and treatment. Malaysian J Pathol 1997;19(1) : 23.

74. Sukumar mukherjee,Alakendu Ghosh,Osteoporosis- The New Global Epidemic. Ashit singleand SD Deodhar. Editors.Rheumatology principles and practice. Jaypee publication: P. 298.

75. S.Agarwal,A.Jain et al.,Correlation of bone mineral density with biochemical markers in postmenopausal women.Indian journal of clinical biochemistry.2009 ;24(3): 262-265.

A STUDY OF S.N- TELOPEPTIDE, A BONE RESORPTIVE BIOMARKER IN POST MENOPAUSAL WOMAN

PROFORMA

NAME OF THE PATIENT : O.P.NO/I.P.NO:

AGE/SEX :

OCCUPATION :

ADDRESS :

COMPLAINTS :

PAST HISTORY :

PERSONAL HISTORY :

FAMILY HISTORY :

DRUG HISTORY :

GENERAL EXAMINATION:

HT: WT: BMI: PR: BP:

SYSTEMIC EXAMINATION:

CVS: RS:

P/A: CNS :

INVESTIGATIONS :

1. S.N-TELOPEPTIDE :

2. S. ESTROGEN :

3. S.ALK. PHOSPHATASE :

4. S. CALCIUM :

5. S.PHOSPHORUS :

6. B. UREA :

7. S. CREATININE :

8. B. SUGAR (R) :

9. BMD :

CONSENT FORM

Dr. P. SUNITHAPRIYA, Post Graduate Student in the Department of Biochemistry, Thanjavur Medical College, Thanjavur is doing a Study on "**A STUDY OF SERUM N-TELOPEPTIDE - A BONE RESORPTIVE BIOMARKER IN POST MENOPAUSAL WOMEN".** The procedures have been explained to me clearly. I understand that there are no risks involved in the above procedures. I here by give my consent to participate in this study. The data obtained here may be used for research and publication.

Signature

Name :

Place :